

**Minutes of the Meeting of the Sub Committee to Review the Implications of the Hon'ble Supreme Court Directions dated 08.05.2007 under the Chairmanship of Dr. B. M. Khadi, Director, CICR, Nagpur held on 24.7.2007**

---

The first meeting of the Sub Committee constituted by the Ministry of Environment & Forests to review the implications of the Hon'ble Supreme Court directions dated 08.05.2007 in Writ Petition 260/2005 filed by M/s Aruna Rodrigues vs. UoI was held on 24.7.2007 under the Chairmanship of Dr. B. M. Khadi, Director, CICR, Nagpur in the Department of Biotechnology, CGO Complex, Lodhi Road, New Delhi.

List of the participants is annexed at **Annexure-1**.

At the outset the Chairman welcomed the members. Before initiating the meeting, he informed the Committee that in the hearing held on 8.5.2007, the Hon'ble Supreme Court order dated 22.9.2006 directing the GEAC to stop all approvals was amended to the extent that the GEAC may accord approval for commercial release of Bt cotton hybrids expressing approved gene events cry 1Ac (MON 531 event), cry 1Ac and cry 2Ab (MON 15985) gene, cry 1Ac (event 1) and cry 1Ab + cry 1Ac GFM. Further, the Hon'ble Supreme Court has also permitted conduct of field trials of GM crops expressing new gene events subject to the following conditions:

1. All trials should have a lead scientist's name with contact details who would be responsible for all aspects of the trials including regulatory requirements.
2. An isolation distance of 200 m would be maintained during field trials.
3. Prior to bringing out the GM material from the green house for conduct of open field trial the Company should submit a validated event specific test protocol at an LOD of at least 0.01% to detect and confirm that there has been no contamination.

However, the GEAC in its 76<sup>th</sup> meeting held on 11.5.2007 was of the view that the direction of the Hon'ble Supreme Court to maintain isolation distance of 200 m during GM crop field trials and a 0.01 % LOD cannot be uniformly applicable for all crops as the nature of pollen flow and level of cross pollination with related species would vary depending on the biology of the crop and the host environment. Accordingly, the GEAC has set up a Sub Committee with the following terms of reference :

- a. To examine the implications of the Hon'ble Supreme Court Order dated 8.5.2007 especially with respect to conduct of field trials and measures to be taken by the GEAC to ensure its compliance.

- b. Feasibility of implementing the 200 m isolation distance during field trials (MLT/LST) of different GM crops.
- c. Feasibility of detection of GM contamination at LOD of at least 0.01%

The Chairman then invited the members to present their views on the conditions stipulated by the Hon'ble Supreme Court. Details of the deliberations are summarized below:

**A. Isolation distance of 200 m to be maintained during GM crop field trials:**

a. Gene flow, a natural phenomenon, to the neighboring fields from other fields of the same crop if they are planted very closely can occur principally due to movement of pollen. In self-pollinated crops, the chances of cross pollination from other sources is much less, as the physiology of the flower would be such that, it would get fertilized only with its own pollen before they are exposed to the pollen from other unrelated species and sources. In the case of cross-pollinated plants, pollen movement from other sources is necessary for fertilization and seed production. In nature, the wind, bees, insects and other pollinators cause the pollen movement. These factors are causing concerns to the seed sector while producing seeds with genetic identity and genetic purity. To maintain genetic identity and genetic purity "The Central Seed Certification Board" of the Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India has published "Indian Minimum Seeds Certification Standards" in July, 1988. The manual provides guidelines for minimum isolation distance to be maintained, minimum permitted cross pollination and maximum genetic purity standards to be maintained for foundation and certified seeds for each crop. These standards are formulated based on nearly one and half decade of research activities in Seed Technology sector. These standards were thoroughly discussed by the Technical Committee of Central Seed Certification Board. Directors of Seed Certification Agencies, Members of Seed Producers and Seed Users from public and private sectors participated in discussion as special invitees and were then approved by the Central Seed Certification Board and Central Seed Committee in a joint meeting. These standards came in to force from Kharif, 1988 for issuing seed certificates.

b. Here the concept is that to keep the seed production fields away from the neighboring fields with a minimum isolation distance, so that the pollen would not cross-contaminate the seed production fields and would be possible to maintain the desired genetic purity. This is a physical containment measures built-in for maintaining the genetic purity. The isolation distances thus vary from crop to crop and within the crop from variety as well as hybrids. In other words, each crop has its unique isolation distance requirement in seed production and certification process.

c. The above concept was adopted by the Government of India while allowing the field trials on transgenic crops. Here, the isolation distances were prescribed to avoid gene flow to the neighboring

fields from the pollen from the transgenic crops, so that there is little probability of genetic crossing from the transgenic field. It may be noted here that, all the transgenic crops are studied for its pollen morphology, pollen viability, pollen flow and other characters and observed that the pollen from transgenic crops is not different from that the corresponding non transgenic crop. By undertaking the pollen flow studies in a scientific manner on the transgenic crops it was further established that the pollen, in general natural conditions, depending on the crop, flow to small distances only and certainly not flow beyond the isolation distances as specified in the guidelines.

d. While permitting the field trials on transgenic crops, the Government has prescribed two types of containment measures to be adopted for restricting the possibility of gene flow to the neighboring crops. These are: a) isolation distances as prescribed in the Indian Minimum Seeds Certification Standards (as physical containment measure) and b) planting non transgenic plants of the same crop up to 2-5 meters width (as pollen trapper rows, depending on the type of crop ) all round the experimental plot with in the isolation distance to trap the pollen coming from the transgenic plants ( as biological containment measures). The combination of physical and biological containment measures are prescribed to ensure the restriction of cross pollination to the highest possible levels.

e. Information on the isolation distance prescribed for each crop in the Indian Minimum Seeds Certification Standards is tabulated in **table 1**:

**Table 1: Adequate biosafety during the conduct of field trials for GM crops**

|    | <b>Crop</b> | <b>Nature of pollination</b> | <b>Pollinating agent (s)</b> | <b>Minimum isolation (m) prescribed in IMSCS@</b> | <b>Isolation (m) recommended by the sub-Committee</b> | <b>Additional biosafety measures recommended</b>   |
|----|-------------|------------------------------|------------------------------|---|---|--|
| 1. | Cotton      | Often cross pollinated       | Insects                      | 50  | 50  | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |

|    |           |       |                         |                        |      |  |
|----|-----------|-------|-------------------------|------------------------|------|--|
| 2. | Rice      | Self  | Wind                    | 200                    | 200  | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 3. | Okra      | Cross | Insects                 | 500                    | 500* | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 4. | Groundnut | Self  | -                       | 3                      | 10   | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 5. | Potato    | Self  | -                       | 5 (tubers)<br>50 (TPS) | 100  | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 6. | Tomato    | Self  | Solitary bee,<br>thrips | 200                    | 200  | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 7. | Brinjal   | Self  | Insects                 | 300                    | 300  | Physical barrier   |

|    |  |            |      |                                  |      |   |
|----|--|------------|------|----------------------------------|------|---|
|    |  |            |      |                                  |      | around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra.   |
| 8. | Castor                                 | Cross      | Wind | 600 (Hybrid)<br>1000<br>(Female) | 1000 | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra.  |
| 9. | Cauliflower & Cabbage***               | Cross      | Bees | 1600                             | 200  | To be harvested at curd maturity stage.<br>Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra. |
| 10 | Mustard & Rapeseed (Oilseed brassicas) | Self Cross | Bees | 50-100                           | 100  | Physical barrier around the crop of ~ 700 gauge thick polythene upto 2 m height or barrier crops like Sorghum, Maize or Bajra.  |

*@Indian Minimum Seed Certification Standards (1988), Central Seed Certification Board, DAC, Ministry of Agriculture, GOI, New Delhi*

*Compendium of proceedings of Technical Committee of CSCB (1986-2004). (Revisions and Amendments).*

*Central Seed Certification Board, DAC, Min. of Agriculture, GOI, New Delhi.*

\*\*\* For seed production, 1600 m shall be maintained.

**B. Submission of a validated event specific test protocol at an LOD of at least 0.01% to detect and confirm that there has been no contamination.**

a. **Since stringent guidelines of maintaining scientifically prescribed isolation distance ( which is more stringent than the 200 metres prescribed by the Hon'ble Supreme Court) and additional biosafety measures are being adopted for field-testing of crops, the possibility of contamination is rare.** Therefore, the LOD may be defined at levels that are practical to use, compatible with the acceptable thresholds that may be defined for labeling purposes and are based on the technical aspects of the analytical methods and the genome size of the crop. Several countries have adopted different thresholds that suit their sensibilities and biosafety (table 2).

**Table 2: Regulations concerning the labeling of GM products in select countries.**

| Country        | Labeling status                 | Threshold %              | Implementing data |
|----------------|---------------------------------|--------------------------|-------------------|
| Australia      | Mandatory                       | 1                        | Dec 2001          |
| New Zealand    | Mandatory                       | 1                        | Dec 2001          |
| Brazil         | Mandatory                       | 4                        | Dec 2001          |
| Canada         | Mandatory                       | -                        | Nov 1994          |
| China          | Mandatory                       | 0                        | July 2001         |
| Czech Republic | Mandatory                       | 1                        | Not available     |
| European union | Mandatory                       | 0.9, 0.5 for food & feed | July 2003         |
| Hong Kong      | Voluntary                       | 5                        | Feb 2001          |
| Israel         | Mandatory                       | 1                        | Not available     |
| Japan          | Mandatory for selected products | 5                        | April 2001        |
| Korea          | Mandatory for selected products | 3                        | June 2001         |
| Malaysia       | Mandatory                       | 3                        | Proposal          |
| Russia         | Mandatory for selected products | 5                        | Sept 2002         |
| Switzerland    | Mandatory                       | 2 or 3 for feed          | Not available     |
| Taiwan         | Mandatory                       | 5                        | Proposal          |
| Thailand       | Mandatory for selected products | 5                        | Proposal          |
| USA            | Voluntary                       | -                        | Jan 2001          |

*Data from Jia 2003 and ISAAA 2005.*

b. The limit of detection cannot be common to all crop species (table 3) since the LOD test is dependent on genome size and the number of transgene inserts per genome. Genome size is fixed for each species (table 4 and table 5) and determines the number of genomes present in a fixed amount of DNA sample being used per PCR test.

**Table 3: Validated LOD tests reported across the globe**

| Crop    | Company  | Event       | Year | Method | Trait                            | LOD%   |
|---------|----------|-------------|------|--------|----------------------------------|--------|
| Maize   | Monsanto | Mon-810     | 2007 | RT-PCR | Insecticide resistance           | <0.1%  |
| Maize   | Novratis | Bt-11       | 2007 | RT-PCR | Insecticide & herbicide tolerant | 0.1%   |
| Maize   | Aventis  | CBH-351     | 2007 | PCR    | Insecticide & herbicide tolerant | 0.1%   |
| Maize   | Pioneer  | DAS-59122-7 | 2006 | RT-PCR | Insecticide & herbicide tolerant | 0.1%   |
| Maize   | Syngenta | Event-176   | 2007 | RT-PCR | Insecticide & herbicide tolerant | 0.1%   |
| Maize   | Monsanto | Ga21        | 2006 | RT-PCR | Herbicide tolerant               | <0.1%  |
| Maize   | Monsanto | Mon836      | 2006 | RT-PCR | Insecticide Resistance           | 0.1%   |
| Maize   | Monsanto | NK603       | 2006 | RT-PCR | Herbicide tolerant               | 0.1%   |
| Maize   | Aventis  | T25         | 2007 | RT-PCR | Herbicide tolerant               | 0.1%   |
| Maize   | Pioneer  | TC1507      | 2006 | RT-PCR | Insecticide & herbicide tolerant | 0.1%   |
| Rice    | Bayer    | LLRICE62    | 2006 | RT-PCR | Herbicide tolerant               | 0.01%  |
| Cotton  | Dow      | 281-24-236  | 2007 | RT-PCR | Insecticide & herbicide tolerant | <0.04% |
| Cotton  | Dow      | 3006-210-23 | 2007 | RT-PCR | Insecticide & herbicide tolerant | <0.04% |
| Soybean | Monsanto | GTS 40-3-2  | 2007 | PCR    | Herbitol                         | <1.0%  |

**Table 4: GM Crop species under various stages of testing in India**

| Crop                 | Scientific name                | C pg | Reference                    |
|----------------------|--------------------------------|------|------------------------------|
| Cotton               | <i>Gossypium hirsutum</i>      | 2.48 | Hendrix & McStewart, 2005    |
| Rice                 | <i>Oryza sativa</i>            | 0.50 | Bennett et al., 1976         |
| Maize                | <i>Zea mays</i>                | 2.73 | Bennett et al., 1976         |
| Pigeonpea            | <i>Cajanus cajan</i>           | 0.88 | Bennett and Smith, 1976      |
| Soybean              | <i>Glycine max</i>             | 1.13 | Greilhuber & Obermayer, 1996 |
| Groundnut            | <i>Arachis hypogaea</i>        | 2.87 | Temsch & Greilhuber, 2000    |
| Mustard              | <i>Brassica juncea</i>         | 1.53 | Verma and Rees, 1974         |
| Tomato               | <i>Lycopersicon esculentum</i> | 1.03 | Bennett and Smith, 1976      |
| Brinjal              | <i>Solanum melongena</i>       | 0.98 | Bennett et al., 1976         |
| Cabbage, Cauliflower | <i>Brassica oleracea</i>       | 0.78 | Olszewska & Osiecka, 1983    |
| Bhendi               | <i>Abelmoschus esculentus</i>  | 1.65 | Bennett et al., 1999         |

**Table 5: Determination of minimum quantity of DNA required in PCR for reliable GM detection For LOD tests.**

| Sample size: eg seeds   |             |                    | <b>30,000</b>                                      |               | <b>10,000</b> |               | <b>3000</b>  |               | <b>1000</b>  |               | <b>300</b>  |               |
|-------------------------|-------------|--------------------|--|---------------|---------------|---------------|--------------|---------------|--------------|---------------|-------------|---------------|
|                         |             |                    | <b>Number of nuclear DNA copies in 200 ng DNA*</b> |               |               |               |              |               |              |               |             |               |
| <b>LOD at 95%</b>       |             |                    | <b>0.01%</b>                                       |               | <b>0.03%</b>  |               | <b>0.1%</b>  |               | <b>0.33%</b> |               | <b>1.0%</b> |               |
| <b>Crop</b>             | <b>C pg</b> | <b>Per nucleus</b> | <b>GM</b>  | <b>Non-GM</b> | <b>GM</b>     | <b>Non-GM</b> | <b>GM</b>    | <b>Non-GM</b> | <b>GM</b>    | <b>Non-GM</b> | <b>GM</b>   | <b>Non-GM</b> |
| Cotton                  | 3.23        | 4.96               | <b>4.0</b>   | 40318.6       | <b>12.0</b>   | 40310.6       | <b>40</b>    | 40282.3       | <b>120</b>   | 40202.6       | <b>402</b>  | 39919         |
| Rice                    | 0.5         | 1                  | <b>20.0</b>  | 199980.0      | <b>60.6</b>   | 199939.4      | <b>200.0</b> | 199800.0      | <b>606.1</b> | 199393.9      | <b>2000</b> | 198000        |
| Maize                   | 2.73        | 5.46               | <b>3.7</b>   | 36626.4       | <b>11.1</b>   | 36618.9       | <b>36.6</b>  | 36593.4       | <b>111.0</b> | 36519.0       | <b>366</b>  | 36264         |
| Pigeonpea               | 0.88        | 1.76               | <b>11.4</b>  | 113625.0      | <b>34.4</b>   | 113601.9      | <b>113.6</b> | 113522.7      | <b>344.4</b> | 113292.0      | <b>1136</b> | 112500        |
| Soybean                 | 1.13        | 2.26               | <b>8.8</b>   | 88486.7       | <b>26.8</b>   | 88468.8       | <b>88.5</b>  | 88407.1       | <b>268.2</b> | 88227.4       | <b>885</b>  | 87611         |
| Groundnut               | 2.87        | 5.74               | <b>3.5</b>   | 34839.7       | <b>10.6</b>   | 34832.6       | <b>34.8</b>  | 34808.4       | <b>105.6</b> | 34737.6       | <b>348</b>  | 34495         |
| Mustard                 | 1.53        | 3.06               | <b>6.5</b>   | 65352.9       | <b>19.8</b>   | 65339.7       | <b>65.4</b>  | 65294.1       | <b>198.1</b> | 65161.4       | <b>654</b>  | 64706         |
| Tomato                  | 1.03        | 2.06               | <b>9.7</b>   | 97077.7       | <b>29.4</b>   | 97058.0       | <b>97.1</b>  | 96990.3       | <b>294.2</b> | 96793.2       | <b>971</b>  | 96117         |
| Brinjal                 | 0.98        | 1.96               | <b>10.2</b>  | 102030.6      | <b>30.9</b>   | 102009.9      | <b>102.0</b> | 101938.8      | <b>309.2</b> | 101731.6      | <b>1020</b> | 101020        |
| Cabbage,<br>Cauliflower | 0.78        | 1.56               | <b>12.8</b>  | 128192.3      | <b>38.9</b>   | 128166.3      | <b>128.2</b> | 128076.9      | <b>388.5</b> | 127816.6      | <b>1282</b> | 126923        |
| Bhendi                  | 1.65        | 3.3                | <b>6.1</b>   | 60600.0       | <b>18.4</b>   | 60587.7       | <b>60.6</b>  | 60545.5       | <b>183.7</b> | 60422.4       | <b>606</b>  | 60000         |

\*All standard LOD tests deploy a maximum of 200 ng DNA per PCR for a 50µl sample. Template DNA quantity above 200 ng generally results in problems with amplification.

c. Further the LOD test is affected by the sampling size (**table 6**) type of matrix present, extraction efficiency, and sensitivity of the test. All these factors are influenced by the biological characteristics of each crop. For example, cotton material contains phenolics and the matrix is not easily accessible for complete extraction of DNA from samples.

**Table 6: Determining Laboratory Sample sizes using binomial distribution**

| <b>Grain number</b> | <b>Probability level based on binomial distribution</b> |            |            |
|---------------------|---|------------|------------|
|                     | <b>90%</b>  | <b>95%</b> | <b>99%</b> |
| 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%  |
| 20000 | 0.01%  | 0.015% | 0.02%  |
| 30000 | 0.005% | 0.01%  | 0.015% |

d. A brief table is presented in this report (**table 7**), which determines the possible LOD values for each of the crops depending on their genome size and the practical feasibility of the test in a standard laboratory.

**Table 7: LOD values derived for the crops being considered for GM testing**

| <b>Crop</b>          | <b>LOD at minimum of 20 GM copies</b><br>(highly specialized labs) | <b>LOD at minimum of 100 GM copies</b><br>(Specialized labs) | <b>LOD (Practical) at minimum of 400 GM copies</b><br>(Moderately specialized labs) |
|----------------------|--|--|---|
| Cotton               | 0.050%   | 0.248%   | 1.000%  |
| Rice                 | 0.010%   | 0.050%   | 0.200%  |
| Maize                | 0.055%   | 0.273%   | 1.092%  |
| Pigeonpea            | 0.018%   | 0.088%   | 0.352%  |
| Soybean              | 0.023%   | 0.113%   | 0.452%  |
| Groundnut            | 0.057%   | 0.287%   | 1.148%  |
| Mustard              | 0.031%   | 0.1535   | 0.612%  |
| Tomato               | 0.021%   | 0.103%   | 0.412%  |
| Brinjal              | 0.020%   | 0.098%   | 0.392%  |
| Cabbage, Cauliflower | 0.016%   | 0.078%   | 0.312%  |
| Bhendi               | 0.033%   | 0.165%   | 0.660%  |

e. Limits of Detection (LOD) is the concentration of the least amount of analyte that can be reliably detected. For DNA-based detection methods, the LOD is dependent on genome size (Van den Eede et al., 2002) and the number of transgene inserts per genome, The LOD test is affected by the sampling size, type of matrix present, extraction efficiency, and sensitivity of the test. To determine whether a matrix alters either the LOD, a comparison can be made between an analyte extracted from

a feed sample with a known content of GM plant and a standard obtained separately from the sample (Stave, 1999). Standards can be produced from *in vitro* methods, such as replication of plasmid DNA or the expression of protein in bacteria, or they may be derived from a single GM plant (total DNA or protein isolated from one plant). Alternatively, a known amount of GM standard can be introduced into isolated DNA or the protein matrix that came from a feed containing non-GM parental plant. Differences between detection or quantification of the recombinant analyte with and without the matrix can then be determined (Stave, 2002). Proper sampling procedures are critical to reliable detection of GM products in feeds. A significant amount of error can arise from collection methods and that error increases as the concentration of GM feed decreases (Gilbert, 1999).

f. A detection methodology to be adopted, the sampling strategy and analysis method should achieve an acceptable balance between sampling error and the cost to attain a confident level of certainty. Any sampling plan should require that the random sample is large enough to represent the entire lot of feed. The allowable GM threshold will affect the sampling regime, with lower threshold limits requiring larger sample sizes to achieve a set confidence level (Gilbert, 1999). The method used to select laboratory samples can also be a significant source of error in the detection of GM feed. Typically, a field sample taken from a large bulk source such as a truck is sub-sampled and then that sample is ground to a fine powder. Depending on the analytical test to be performed, a sub-sample from this powder or the entire powder is used for analysis as a test sample. Sensitive methods such as polymerase chain reaction (PCR), 100–200 mg of test sample may be enough material for analysis. Only a sample of the total extracted DNA is used in PCR. Typically, this amount is 100–200 ng of DNA. The initial sub-sample size and number of repeats should be determined with consideration of the precision of each method used. Hubner et al. (2001) described a laboratory sample scheme for the detection of GM maize and soybean by quantitative competitive PCR (QC-PCR) and real-time PCR (RT-PCR) methods. When DNA is isolated from a sample, other factors, including plant sub-strates, feed additives, or reagents used in extraction procedures, can be co-purified. The effects of these factors on analytical tests, if any, are called matrix effects (Stave, 1999). Typically, impurities in a plant or DNA matrix have adverse effects on sample analyses. Impurities are usually more critical to DNA analysis than to protein analysis, and can significantly affect the efficiency of detection. Plant polysaccharides and polyphenolics can inhibit the PCR or degrade DNA (Wilson, 1997; Demeke and Adams, 1992). Hexadecyltrimethylammonium bromide (CTAB) and isopropanol are examples of chemicals commonly used in the extraction of DNA that are known to inhibit PCR at concentrations greater than 0.05 mg/ml (w/v) and 0.01 ml (v/v), respectively (Peist et al., 2001). It is important to test for these effects when standardizing procedures, as they can affect the limit of detection (LOD). The DNA content of the unreplicated haploid complement, known as the 1C value, can be used as an intrinsic factor that relates the number of genome copies to amount. As previously explained, up to 36 697 copies would be present in a typical 100ng analytical sample of *Zea mays*, given the 1C value of 2.725pg (Bennet et al., 2000). This implies that for such a sample, the presence of a single GMO copy

would represent a w/w percentage of 0.0027% (Cochran, 1977). For a 100ng sample, therefore, this becomes the lowest concentration theoretically possible; absence of detection in a lower concentration laboratory sample would thus be a statistical artefact, and not a valid result. If the laboratory sample is large (say, 50µg), the selection of the analytical sample (100ng) can be made according to simple random sampling procedures, with replacement (Forthofer and Sul Lee, 1995). However, even in conditions of perfect homogeneous preparation of the laboratory sample, "sampling errors" will occur. In particular, when the concentrations concerned are low, the sampling error becomes (proportionally) larger. Furthermore, given that this is purely sampling error, degradation in this result can be expected due to the imperfect behaviour of a real analytical system.

g. In the case of PCR, the expected number of GM DNA copies actually present in the sample should be sufficient to respect the LOD of the test: In general, this will be  $\geq 20$  copies, and  $>100$  copies for LOQ (Kay & Van den Eede, 2001). However, studies showed that the practical detection limit is significantly (20x) lower (Jankiewicz et al. 1999) in multi-location tests to validate the LOD. Based on the 'C' value of the genome, and the requirement of  $\geq 20$  copies of GM template DNA per PCR test, following are the theoretical and practical LOD values derived for the crops being considered for GM testing in India.

h. It is possible to calculate, using the well-understood cumulative distribution function for the binomial distribution (Biotecon Diagnostic GmbH., 2000), the probable range of number of GM copies that would be "sampled" in a single-step procedure, i.e. from a (large) laboratory sample of "known" low concentration (0.1% GMO) into a series of 100ng analytical samples. While on average, the analytical samples should contain 36.7 GMO copies; in fact the number of GMO copies would range from 25 to 48, with a 94.3% confidence level. Thus the actual concentration that would be observed in a single sample, with an approximate 95% confidence level, would range from 0.068% to 0.131%; the probability of sampling exactly 36 GMO copies in a single analytical sample is only 0.066 %. With lower concentrations, the situation is more critical. For a laboratory sample of 0.01% concentration, the 100ng analytical sample would vary between 0.0027% and 0.0191% nearly 95% of the time (Kay et al., 2001). These calculations obviously refer to a "best possible" result, since they assume a single sampling step and a perfect analytical system. When undertaking a dilution series, it is important to note that the assumption of sampling with replacement may no longer be valid, since the number of copies available becomes strictly finite. Indeed, the number of copies used to prepare subsequent dilutions heavily influences the sampling error associated with the series. Consequently, the preparation of any dilution series must be undertaken in such a way so as to minimize this bias; ideally, dilutions should be made from the primary laboratory sample. The classical solution to the issue of sampling error is to undertake repetitions, and/or use appropriately sized (i.e., larger) analytical samples. Therefore it is recommended that in the construction of a dilution series - for example for determination of Limit of Detection of a method, or for the generation of standard curves - that the nominal number of GMO copies in the weakest dilution of analytical sample should be set to

around 20, thus providing good statistical probability that all repetitions contain relevant DNA. However, we are aware of important studies that seem to draw conclusions without such safeguards, despite explicitly working with copy numbers. In conclusion, there is insufficient acknowledgement in the domain that repeated analytical samples drawn from a "homogenized" laboratory sample would not have identical proportions of GMO/non GMO copies.

### **Recommendations:**

In light of the above observations, the Committee recommended the following:

1. Scientifically, a uniform isolation distance is not tenable as the nature of the pollen flow and level of cross pollination depends on the biology of the crop and the host environment it is being cultivated. Therefore, each crop has its own unique isolation distance. It is recommended that the isolation distance prescribed under the Indian Minimum Seed Certification Standards manual may be followed. In addition to the prescribed isolation distance, additional biosafety measures as indicated **table 1 (Column 7)** may be prescribed on a case to case basis. The combination of physical and biological containment measures prescribed by the regulatory agencies is adequate to ensure the restriction of cross pollination to the highest possible levels.
2. In view of the stringent guidelines of maintaining scientifically prescribed isolation distance (which, for several crops, is more than the 200 m isolation distance prescribed by the Hon'ble Supreme Court) and additional biosafety measures prescribed during field testing of GM crops, the possibility of contamination is rare (**refer table 1**). Therefore, the requirement for a validated protocol of 0.01% LOD may be dispensed with.
3. Prior to the commercial release/ export requirements, the LOD may be decided at levels that are practical to use, compatible with the acceptable thresholds that may be defined for labeling purposes and are based on the technical aspects of the analytical methods available in the international laboratories and the genome size of the crop. Thus the arbitrary requirement for provision of a detection method with an LOD of 0.01% at the time of field trial may be dispensed with.

The meeting ended with a vote of thanks to the Chair.

\*\*\*\*\*

**Anneuxre -1****List of participants who attended the meeting of the Sub Committee on 24.7.2007.**

| <b>S.No.</b> | <b>Name and Designation</b>   |
|--------------|---|
| 1.           | Dr. B. M. Khadi, Director, CICR, Nagpur   |
| 2.           | Dr. K. K. Tripathi, Adviser, Department of Biotechnology, New Delhi.                                |
| 3.           | Dr. Anand Kumar, Head (NRC), IARI, New Delhi  |
| 4.           | Dr. Malvika Dadlani, Head, Division of Seed Science and Technology, Min. of Agriculture, New Delhi. |
| 5.           | Prof. Govindraj Hegde, Assistant Professor, National Law School, Bangalore                          |
| 6.           | Dr. R. Warriar, Director, MoEF, New Delhi.  |
| 7.           | Ms. Madhu Gupta, RO, MoEF, New Delhi.   |

**Special invitees**

| <b>S.No.</b> | <b>Name and Designation</b>  |
|--------------|--|
| 1.           | Dr. Rakesh Tuli, Director, NBRI, Lucknow.                                    |
| 2.           | Dr. V. S. Reddy, Scientist, ICGEB, New Delhi                                 |
| 3.           | Dr. Vibha Ahuja, Dy. General Manager, Biotech Consortium India Ltd., N.Delhi |
| 4.           | Dr. K.R. Kranthi, Scientist, CICR, Nagpur.                                   |