



Health Canada

Santé Canada

# **INDUSTRIAL HEMP**

# **TECHNICAL MANUAL**

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# BASIC ANALYTICAL PROCEDURE FOR THE DETERMINATION OF $\Delta^9$ -TETRAHYDROCANNABINOL (THC) IN INDUSTRIAL HEMP

## 1. PURPOSE

To measure THC content in the foliage and involucral leaves of hemp inflorescences. (Specimens are prepared with a view to obtaining foliage and involucral leaves as test material.)

## 2. SCOPE

The THC content is measured only on samples which have been prepared by the Method for Sample Handling and Preparation (TPP-BDS-003), from field samples which have been obtained according to the Procedure for Field-sampling of Hemp Stands (TPP-BDS-002).

## 3. BACKGROUND INFORMATION/RATIONALE

THC concentration is determined by means of an analytical method which meets the requirements of the performance standard published in Appendix B to this Manual.

## 4. REFERENCED DOCUMENTS

1. Meijer, E.P.M.; van der Kampf, H. H and van Eeuwijk, F. A., Euphytica, 62 (1992), p. 187 - 200.]

2. LeBelle M. and Savard C., Bureau of Drug Research, Health Canada, 1992  
(may be found at the Health Canada website, [www.hc-sc.gc.ca/hpb-dgps/therapeut](http://www.hc-sc.gc.ca/hpb-dgps/therapeut)).

## 5. SAFETY

It is essential that all safety and security procedures in place in the laboratory be followed rigorously. Because of the nature of the samples, materials for analysis should be dealt with as if they were forensic

specimens.

## 6. EQUIPMENT

Capillary gas-chromatograph.

## 7. REAGENTS

### Hexane (reagent-grade)

Standard solution consisting of 1 mg THC in 1 ml of methanol

### Internal standard solution

Mixture consisting of hexane (reagent-grade) and internal standard.  
(Referenced documents 1 and 2).

### Calibrating solutions

Calibration solutions equivalent to 2.5%, 1%, 0.5%, 0.25% and 0.1% THC should be prepared in internal standard solution.

## 8. PROCEDURE

The requisite dry-mass determination is to be conducted as a double measurement. Approximately 3 g of leaf powder are accurately weighed , and dried for four hours at 103° C in the oven.

### 8.1 Extraction

Measures must be taken using at least two aliquots of test material from a specimen (double measurement).

Approximately 100 mg of leaf powder are accurately weighed into a centrifuge tube. Then, 5 ml of internal standard solution are added. The THC is extracted in an ultrasonic bath within 20 minutes.[depth of submergence of the tubes: The solution in the tubes must be at least completely submerged in the bath fluid.] The extracts are clarified by centrifugation for 5 min. at 3,000 rpm. The clear extracts are transferred into the gas chromatography vials.

## 8.2 Gas chromatography

Gas chromatography is to be carried out on a capillary instrument with autosampler, split/splitless injector and flame ionization detector. A suitable column is cross-linked 5% phenyl-methyl-silicone, [ ID: 0.32 mm, length: 30m, film thickness: 0.25 µm]. Using a split ratio of 30:1 and nitrogen as the carrier gas, a suitable flowrate is 20 ml/min for a temperature programme such as: 240° C for three minutes, then 15° C increments/minute to 280° C, then 5 minutes at 280° C. The injection volume is 1µL.

## 9. **QUALITY CONTROL/RESULTS VERIFICATION**

### Results:

Results are calculated as grams of Δ9-THC per 100 g of dry weight, in percent, to two decimal places. The coefficient of variation of the results must be equal to or better than that cited for intra-assay variation in Appendix B to this Manual.

## 10. **METHOD VALIDATION**

Validation of the analytical method in the hands of the actual analyst performing the analysis is mandatory. The validation procedure must be performed as specified in the laboratory's SOP for validation of analytical methods. Furthermore the analytical procedure must be demonstrated to meet the performance standard in Appendix B to this manual before being used for any analyses.

## PROCEDURE FOR FIELD-SAMPLING OF HEMP STANDS

### 1. PURPOSE

Samples are taken to obtain specimens whose  $\Delta 9$ -THC content is then determined. The ascertained values should constitute the average THC content of the hemp stand in the area sampled. The ascertained content is then compared with the maximum content of 0.3% tetrahydrocannabinol in industrial hemp laid down in these Regulations.

The procedure described can also be used when it is necessary to develop specimens for an arbitrary number of hemp stands for subsequent THC determination.

### 2. SCOPE

Limiting the sample to only 30 plants requires homogeneous composition of the stand being sampled. If such is the case, the area of the stand sampled is irrelevant. Since it is very difficult for the examiner to ascertain with certainty whether the stand is homogeneous (variability of varieties, lack of overview), the procedure for sampling is laid down.

THC content of the leaves surrounding the seeds can be expected to peak as the seeds begin to ripen. The time at which samples are taken is therefore important with a view to monitoring compliance with a threshold value.

Having regard to current procedures, the storage of the samples to be prepared as well as their normal shipment requires that the sample material be dried.

### 3. BACKGROUND INFORMATION/RATIONALE

Sampling is done in a manner that will provide appropriate analytical specimens.

## 4. TERMINOLOGY/DEFINITIONS

### 4.1. Sampling unit

Inflorescence of the hemp plant

### 4.2. Composite sample

Total number of hemp inflorescences taken from the stand of an area

### 4.3. Test specimen

The number of inflorescences (minimum of 30) taken arbitrarily from the composite sample.

### 4.4. Set-aside specimen

The number of inflorescences (minimum of 30) taken arbitrarily from the composite sample [This specimen is to be kept by the hemp grower.]

### 4.5. Area

The growing area (as reported and uniquely identified by survey coordinates and legal address) from which the stand is represented by the present specimen.

## 5. SAFETY

It is essential that the sampling procedures described in this Official Method be performed only by persons trained in the procedures. Such training would be expected to include details on appropriate safety measures to be used in the field.

## 6. EQUIPMENT/MATERIALS

Garden shears; composite sample container (carrying bag); sacks made from material known to be free from THC, (approximately 75 x 53 cm); sealing material and pliers; labels and/or tags for specimens; sampling record (form).



## 7. PROCEDURE

### 7.1 Sampling procedure

The tester shall estimate average plant height. The tester shall ascertain the appearance of the stand by describing stand density, the condition of the plants and the degree of maturity of the inflorescences. Then, the tester shall make a decision regarding homogeneity of the stand and thus regarding the suitability of the area for sampling from the perspective of the desired monitoring and statistical requirements.

Observations shall be recorded in the sampling record. If the area is only part of a growing area, the location of the sampled area shall be illustrated by means of a sketch.

### 7.2 Time of sampling

Sampling shall commence as the seed begins to mature, i.e. when the first seeds of 50% of the plants are resistant to compression.

### 7.3 Making rounds through the area

To collect samples, the tester shall always walk at right angles to the rows of plants. The tester shall always walk toward two points opposite each other.

### 7.4 Sampling

While walking through the area being sampled, the tester shall cut off at least 60 inflorescences at random but expedient distances.

a) The entire, fruit-bearing part of the plant shall be used as a sample.

b) The cut shall be made underneath the inflorescence (normally the top one-third of the plant).

## 7.5 Test specimens and set-aside specimens

Immediately after the sample is taken, samples are taken arbitrarily from the composite sample and divided up into two sacks. Once they have been divided up, there must be at least 30 samples in each sack, with the number of single specimens the same in each. The sacks are tied up, sealed and labelled. One sack shall be identified as containing test specimens, and the other as containing set-aside specimens. The set-aside specimens shall be kept by the hemp grower. The test specimens are to be used to determine  $\Delta 9$ -THC content.

## METHOD FOR SAMPLE HANDLING AND PREPARATION

### 1. PURPOSE

Monitoring THC in hemp; "handling specimens"

### 2. SCOPE

It is essential that specimens to be submitted for laboratory analysis be prepared only by this Official Method. Because of the variable nature of the substrate, deviations from this Method are not permitted. It is expected that the initial part of this procedure will be carried out by the person responsible for collecting the field samples. At the indicated part of Section 6.2 the samples must be shipped to the testing laboratory.

### 3. BACKGROUND INFORMATION/RATIONALE

The specimens as obtained from the field consist of the inflorescences which once obtained, must be dried. Drying (in accordance with Procedure 1 or 2) must be begun no later than 12 hours after the samples have been taken.

### 4. SAFETY/SECURITY

It is essential that, at all times, specimens be considered as forensic material, and their security before, during and after the performance of the procedures described in this Official Method must be of paramount importance.

### 5. EQUIPMENT/MATERIALS

Rotary screen, diam.20 cm; screens, 3.15mm mesh size and 2mm mesh size; screen deck; lid; brush; pestle approx. 10 cm long; vacuum cleaner; paper bags (sacks); centrifugal grinder with 0.5mm sieve insert with rotor and lid.

## 6. PROCEDURE

### 6.1. Drying

**Procedure I :** The plant parts, loosely packed into the specimen sacks, are dried in these sacks on racks in an oven (drying chamber), with circulating air generally heated to 35 °C for 10 to 12 days. The specimens (with residual moisture content of 15%) are stored in the dark at a temperature of no more than 20° C until they are used. It is imperative that the specimens be stored loosely.

**Procedure II :** The plant parts, loosely packed into the specimen sacks, are dried in the sacks, hung freely in a dark, well-ventilated room at temperatures up to 35 °C. Duration of drying depends on conditions (eg, air exchange, temperature, initial moisture content of the material).

When product moisture ranges from 15 - 20%, specimens can be stored and are ready for processing. Under favourable conditions, product moisture content drops to 8 - 13%.

### 6.2. Preparation

Separation and reduction: The seeds, their involucre leaves and foliage leaves are rubbed off by hand, separated from the stems and collected in a container, such as an aluminum plate (rectangular). Laboratory gloves are worn to protect the hands. Loss of material, especially fine granular components, is to be avoided. Once the plant parts have been processed, the coarse stem parts are separated from the remaining material by sorting.

The mixture, consisting of stem parts, fruits and leaf parts, is ready to be shipped to the testing laboratory at this point. After receipt at the laboratory the sample is treated as follows. It is poured in succession on to a screen, 3.15mm mesh size and a screen, 2mm mesh size, with the screen then moved by hand in order to separate the fraction consisting of foliage and involucre leaves from the stems and seeds. If necessary, a pestle may also be used for the separation process. The pestle is to be cleaned after each use. The stems and seeds are to be discarded.

The fraction consisting of foliage and involucre leaves is ground in a mill with a 0.5 mm round-hole screen insert. The mill must be cleaned after each use (e.g. by air suction). The leaf powder may be stored in paper containers until gas chromatographic analyses are conducted. Cannabinoid content of the specimens does not change over a period of approximately six weeks when kept in darkness at a temperature of not more than 20°C.

## BASIC METHOD FOR THE DETERMINATION OF THC IN HEMPSEED OIL

### 1. PURPOSE

To measure the THC content in oil obtained by crushing the ripened fruits (seeds) of *Cannabis sativa*.

### 2. SCOPE

Because the oil may be intended for internal use as a food product, it is important that the level of THC allowed be kept at an extremely low level. Laboratories must validate the analytical procedure actually used, and must demonstrate that it meets the requirements of the performance standard published in Appendix B to this Manual.

### 3. BACKGROUND INFORMATION/RATIONALE

In theory the ripened seeds of *Cannabis* contain no detectable quantity of THC. However, because of the nature of the material, it is almost impossible to obtain the seeds free from extraneous THC in the form of residues arising from other parts of the plant which are in close proximity to the seeds. Although it is required for the seeds to be cleaned before any subsequent use, the resinous nature of some of the material makes complete cleaning extremely difficult.

### 4. REFERENCED DOCUMENTS

[Method according to C. Giroud and L. Rivier, *Toxicorama*, 7 (4), 15 - 22 (1995)]

### 5. SAFETY

It is essential that all safety and security procedures in place in the laboratory be followed rigorously. Because of the nature of the samples, materials for analysis should be dealt with as if they were forensic specimens.

## 6. EQUIPMENT/MATERIALS

As specified in the literature reference.

## 7. REAGENTS

As specified in the literature reference.

## 8. PROCEDURE

8.1 An aliquot of hempseed oil (200mg), containing THC-D3 (50ng/200mg) as internal standard, is saponified using potassium hydroxide/water/ethanol (1:2:20, w/w/w) for 2 hours at 70°C under nitrogen. After addition of 3ml of water, the THC is extracted using 3 successive portions of 3ml of petroleum ether/diethyl ether (1:1, v/v). The organic solutions are combined and evaporated under nitrogen.

8.2 The THC is quantified following formation of a methyl derivative, according to the following procedure.

After addition of 400µl of dimethylsulfoxide (DMSO) and 20µl of tetrabutylammonium hydroxide (TBAH, 55 - 60% aqueous solution) to the residue from the evaporation, agitation on a vortex-mixer at room temperature for 2 minutes, the extracts are methylated using 100µl of iodomethane (20 minutes at 30°C). The extracts are then acidified with 700µl of hydrochloric acid (0.1M) and the methylated derivative is extracted into 2ml of isoöctane. After centrifugation, the organic layer is removed and evaporated under nitrogen. The residue is taken up into 70µl of isoöctane, and 1µl is injected into the GC-MS.

8.3 A multipoint calibration curve must be obtained using grape-seed oil (or other equivalent oil free from THC) containing a series of amounts of THC (for example, from 10 to 250 ng/20mg of oil). Analyses are performed each time on 4 aliquots of each specimen of hempseed oil, of which two are tenfold dilutions using the same oil as used to prepare the calibration curve.

8.4 The methylated THC is quantified by GC-MS operated in the SIM mode. The ions at m/z 328 and 331, representing respectively the

base peaks from methylated THC and THC-D3, are used for the quantification.



## **9. QUALITY CONTROL/RESULTS VERIFICATION**

Results are calculated as microgrammes of delta9-THC per gramme of hempseed oil. The average is calculated from each pair of single measurements. The permissible limit for test error (ie, permissible difference between the individual values of a double measurement) under repetitive conditions is 0.03% absolute. If there is a greater difference between two single values, another measurement must be taken.

## **10. METHOD VALIDATION**

Validation of the analytical method in the hands of the actual analyst performing the analysis is mandatory. The validation procedure must be performed as specified in the laboratory's SOP for validation of analytical methods. Furthermore the method must be demonstrated to meet the performance standard in Appendix B to this Manual before being used for any analyses.

## SAMPLING OF NON-VIABLE CANNABIS SEED FOR VIABILITY TESTING

### 1. PURPOSE

To obtain a representative sample of a lot of non-viable cannabis seed, for submission to a laboratory for viability testing

### 2. SCOPE

Sampling for viability testing is to be conducted on all lots of non-viable cannabis seed.

### 3. BACKGROUND INFORMATION/RATIONALE

A sample is obtained from the seed lot by taking small portions at random from different positions in the lot and combining them. This sample is thoroughly mixed and a smaller sample is drawn for submission to the testing laboratory. The method described here is extracted from the International Rules for Seed Testing (ISTA, 1996).

### 4. REFERENCED DOCUMENTS

ISTA, 1996. International Rules for Seed Testing. Seed Science and Technology, Volume 24, Supplement, Rules, 1996.

### 5. TERMINOLOGY/DEFINITIONS

#### 5.1 Primary sample

A small portion taken from one point in the lot.

#### 5.2 Composite sample

The combined and mixed primary samples taken from the lot.

5.3 Submitted sample

The sample submitted to the testing laboratory. The submitted sample may be either a subsample of or the whole composite sample.

5.4 Sealed

Sealed means that the containers in which the seed is held are closed in such a way that they cannot be opened to gain access to the seed and closed again without either destroying the seal or leaving evidence of tampering.

5.5 Seed

For the purpose of this document, 'seed' refers to the achene of *Cannabis sativa* L., irrespective of its intended end use, and whether or not it is viable.

5.6 Recognized laboratory

A seed testing laboratory that is accredited by the Canadian Food Inspection Agency (CFIA), or a laboratory accredited by the International Seed Testing Association (ISTA) to issue International Seed Lot Certificates.

## 6. EQUIPMENT/MATERIALS

Stick or sleeve type trier: A hollow brass tube inside a closely fitting outer shell or sleeve which has a solid pointed end. The tube and sleeve have open slots in their walls so that when the tube is turned until the slots in the tube and sleeve are in line, seeds can flow into the cavity of the tube, and when the tube is given a half turn the openings are closed. Exact dimensions are not critical, but for bag sampling a trier 762 mm in length with an outside diameter of approximately 13 mm should be suitable. A bin sampler is of similar construction but much larger, ranging up to 1600 mm in length and 38 mm in diameter.

Nobbe trier for bag sampling: A pointed tube long enough to reach the centre of the bag, with an oval hole near the pointed end. The total length is approximately 500 mm, including a handle of about 100 mm and a point of about 60 mm. An internal diameter of about 10 mm should be sufficient. This trier is not suitable for bin sampling.

## 7. PROCEDURE

7.1 Sampling should be carried out by persons trained and experienced in seed sampling. The sampler shall be independent of any commercial interest which might influence the sampling duties being carried out. The national, provincial or state department of agriculture may be able to advise on who can provide this service.

### 7.2 Size of lot

The lot shall not exceed 10,000 kg, subject to a tolerance of 5%. Larger lots shall be subdivided into lots not larger than 10,000 kg, each of which shall be identified by a separate lot designation.

### 7.3 Containers

The lot shall be in containers which are self-sealing, sealed (or capable of being sealed) and labelled or marked for identification by a unique lot designation.

### 7.4 Marking and sealing the lot

At the time of sampling, all containers must be labelled or marked to show a unique lot identification corresponding to the lot identification which will appear on the Certificate of Analysis. The container shall be sealed, or seen to be sealed by the sampler, or be of a self-sealing type. A container shall be regarded as sealed if it is apparently impossible to open without either destroying the seal or leaving evidence of tampering. No sampled lot, or part of a lot, may be left unsealed.

### 7.5 Apparatus

Each stage of sampling the lot shall be performed using appropriate equipment. Such equipment is described in Section 8.

## 7.6 Sampling technique

**Stick or sleeve type trier:** The trier may be used vertically or horizontally. However, when used vertically it must have partitions dividing the instrument into a number of compartments to ensure representative sampling. Using it either vertically or horizontally, the trier in the closed position is inserted diagonally into the bag or container, then opened and turned or agitated to allow it to fill completely. Thereafter it is closed, withdrawn and emptied into a suitable container. If the trier has been inserted through sack walls of coarsely woven jute or similar material, after removal the point of the trier should be run across the hole a couple of times to pull the threads together and close the hole. Holes in paper bags which have been punctured can be closed with an adhesive patch.

**Nobbe trier:** This trier is not suitable for bin sampling. The trier is inserted gently into the bag, pointing upwards at an angle of about 30° to the horizontal, with the hole facing downwards until it reaches the centre of the bag. The trier is then revolved through 180°, bringing the hole to face upwards, and is withdrawn with decreasing speed so that the quantity of seed obtained from successive locations increases progressively from centre to side of the bag.

**Hand sampling:** Use of a trier is the preferred method, but if none is available hand sampling may be used. It is difficult by this method to sample deeper than about 400 mm, so it may be necessary for the sampler to request that some bags be fully or partially emptied to facilitate sampling, and then refilled.

## 7.7 Sampling intensity

For seed lots in bags (or other containers of similar capacity that are uniform in size):

Up to 5 containers:	Sample each container and always take at least 5 primary samples.
6-30 containers:	Sample five containers or at least one in every three containers, whichever is the greater.
31-400 containers:	Sample 10 containers or at least one in every five containers, whichever is the greater.

401 or more containers:                      Sample 80 containers or at least one in every seven containers, whichever is the greater.

When sampling seed from any other kind of container or from streams of seed entering containers the following sampling intensity should be used:

<u>Lot size</u>	<u>Number of primary samples to be taken</u>
Up to 500 kg	At least 5
501-3,000 kg	One for each 300 kg, but not less than five.
3,001-10,000 kg	One for each 500 kg, but not less than 10

7.8 Weight of sample submitted to the laboratory: Minimum 100 g.

7.9 Taking primary samples

Primary samples of approximately equal size shall be taken from each container sampled, or from each place sampled in the container. The containers shall be selected at random throughout the lot and primary samples drawn from top, middle and bottom of containers, but not necessarily from more than one position in any container. When the seed is in bulk or in large containers, the primary samples shall be drawn from random positions and depths. Seed may be sampled as it enters the containers, provided that the instrument uniformly samples the cross section of the seed stream. It may be operated either under manual or automatic control.

7.10 Obtaining the composite sample

If the primary samples appear uniform they shall be combined to form the composite sample. If they are not uniform, the sampling operation shall be terminated.

7.11 Obtaining the submitted sample

The submitted sample shall be obtained by thoroughly mixing the composite sample and drawing off sufficient seed (100g minimum) to submit to the laboratory.

#### 7.12 Dispatch of submitted sample

Each submitted sample shall be marked with the same identification mark as the lot. The sample shall be sealed. The sample should ordinarily be packed in bags of jute, other cloth material or paper; they shall not be packed in a moisture proof container. Samples shall be dispatched by the sampler to the seed testing laboratory without delay and shall never be left unattended in the owners's premises or with unauthorized persons. If dispatch of the sample is unavoidably delayed, the sample shall be stored in a cool well-ventilated room in such a way that changes in the quality of the seed are minimized. The testing laboratory must be a recognized laboratory.

## VIABILITY TESTING OF NON-VIABLE CANNABIS SEED

### 1. PURPOSE

To assess the germination capacity of non-viable cannabis seed to determine if the seed lot has been rendered non-viable.

### 2. SCOPE

Viability testing is to be conducted on all lots of non-viable cannabis seed.

### 3. BACKGROUND INFORMATION/RATIONALE

The method described here is extracted from the International Rules for Seed Testing (ISTA, 1996).

### 4. REFERENCED DOCUMENTS

ISTA, 1996. International Rules for Seed Testing. Seed Science and Technology, Volume 24, Supplement, Rules, 1996.

### 5. TERMINOLOGY/DEFINITIONS

#### 5.1 Abnormal seedlings

Seedlings which do not show the potential to develop into a normal plant due to significant defects of the root, hypocotyl, cotyledons or epicotyl, such as decayed, split, broken, stunted, etc.

#### 5.2 Dead seeds

Seeds which at the end of the test period have not produced any part of a seedling. Seeds with swollen embryonic tissue due to simple imbibition are classified as dead.





5.3 Germination

The emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil.

5.4 Non-viable Cannabis seed

Seed or viable grain which has been steam sterilized for at least 15min., and which has been subsequently shown to be incapable of germination.

5.5 Normal seedlings

Normal seedlings show the potential for continued development into satisfactory plants. They may have some minor defects such as superficial decay, healed lesions, etc.

5.6 Pure Seed

The following structures shall be considered pure seed, even if immature, undersized, shrivelled, diseased or germinated, provided they can be identified as Cannabis sativa:

- i. Achene, unless it is obvious that no seed is present;
- ii. Piece of achene larger than one-half the original size, unless it is obvious that no seed is present;
- iii. Seed, with the pericarp/testa partially or entirely removed;
- iv. Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

5.7 Authorized laboratory

A seed testing laboratory that is accredited by the Canadian Food Inspection Agency (CFIA), or a laboratory accredited by the International Seed Testing Association (ISTA) to issue International Seed Lot Certificates.

5.8 Sealed

Sealed means that the submitted sample container is closed in such a way that it cannot be opened to gain access to the seed and closed again without either destroying the seal or leaving evidence of tampering.

5.9 Viable

Capable of germinating.

## 6. EQUIPMENT/MATERIALS

### 6.1 Germination substrate

Paper normally manufactured specifically for seed germination testing is used. It should be equivalent to standard weight (38 lb) germination paper supplied by the Anchor Paper Co., Minneapolis, MN, USA, normally in sheets of approximately 38cm x 25cm. Exact specifications are not critical, but the paper must be free of fungi, bacteria and toxic substances which might interfere with the growth or evaluation of seedlings. The paper should possess sufficient strength to enable it to resist tearing when handled during the test. It should have the capacity to hold sufficient water for the whole of the test period, and it should have a pH value within the range 6.0 - 7.5.

### 6.2 Germination chamber

The chamber must be capable of maintaining a constant temperature of 20°C ±1° for 16 hours and 30°C ±1° for 8 hours. The changeover between temperatures should be accomplished in one hour or less. Temperature should be uniform throughout the chamber.

### 6.3 Containers

The tests must be enclosed in moisture-proof containers to allow maintenance of substrate moisture throughout the test period.

## 7. REAGENTS

### 7.1 Water

It is preferable to use distilled or de-ionized water to moisten the germination paper substrate. It must be reasonably free from organic or inorganic impurities and have a pH value within the range 6.0 - 7.5.

## 8. PROCEDURE

### 8.1 Receipt of submitted sample

The sample must be sealed and not show any evidence of tampering.

## 8.2 Working sample

The submitted sample is well mixed, then four hundred pure seeds in replicates of 100 are counted at random.

## 8.3 Planting

Sufficient sheets of germination paper are moistened until their wet weight is approximately three times that of their dry weight. For each replicate, the seeds are evenly spaced on two sheets then covered with a single sheet. The paper is carefully rolled just tight enough to hold the seeds, then placed in an upright position. Four such replicates of 100 seeds each are planted. The upright rolls are placed in a moisture-proof container and transferred to a germination chamber set for alternating temperatures of 20°C (for 16 hours) and 30°C (for 8 hours).

## 8.4 Duration of the test

The test replicates remain in the germination chamber for 7 days after planting.

## 8.5 Evaluation

At the end of the test period, the paper is unrolled and each seed and seedling observed and classified as normal seedling, abnormal seedling or dead seed as defined in Section 6.

## 8.6 Calculation and expression of results

The result of the germination test is calculated as the average of four 100 seed replicates. It is expressed as a percentage by number of normal and abnormal seedlings and dead seeds. The percentage is calculated to the nearest whole number. The sum of the percentage of normal and abnormal seedlings and dead seeds must be 100.

## 8.7 Reporting results

The report should include the seed lot number, and a reference to the method used for the test.



8.8 Interpretation of results

For the lot to be considered non-viable, there must be 0% normal seedlings and a maximum of 5% abnormal seedlings.

**9. QUALITY CONTROL/RESULTS VERIFICATION**

Laboratories conducting this testing must be authorized by the Minister and accredited for seed testing by the Canadian Food Inspection Agency, or by the International Seed Testing Association.

## **APPENDIX A**

### **ACCREDITATION AND QUALIFICATION REQUIREMENTS FOR LABORATORIES, AND FOR SAMPLING AND TESTING PERSONNEL**

#### **Field sampling of hemp stands**

Must be conducted by provincially designated professional or technical agronomists (or equivalent for those provinces having no such designation process), with experience in crop production and sampling, or by pedigreed-seed crop inspectors recognized by the Canadian Seed Growers Association.

#### **Sampling of seed for viability testing**

Must be performed by seed analysts or graders accredited under the Seed Laboratory Accreditation programme of the Canadian Food Inspection Agency (CFIA).

#### **Testing of seed for viability**

Must be performed in a laboratory accredited under the Seed Laboratory Accreditation programme of the CFIA.

#### **THC testing of plant material and hemp oil**

Must be performed in a laboratory authorized by the Minister and accredited for this activity by a recognized accrediting body, or equivalent. The laboratory must ensure that staff engaged in this testing are specifically trained for the purpose.



## APPENDIX B

### Analytical Performance Standards

<u>Item no.</u>	<u>Column I</u> Parameters	<u>Column II</u> Concentration of THC in industrial hemp, other than its derivatives or products containing those derivatives	<u>Column III</u> Concentration of THC in derivatives of industrial hemp, or products containing those derivatives
1.	Minimum limit of detection	0.1% (w/w)	4.0 µg/g
2.	Minimum limit of quantification	0.1% (w/w)	4.0 µg/g
3.	Intra-assay precision	C.V. (is less than or equal to) 10% at 0.3% (n=8)	C.V. (is less than or equal to) 10% at 10.0 µg/g (n=8)
4.	Linear range	r <sup>2</sup> (is less than or equal to) 0.98 in the range of 0.1% to 1.0% (w/w)	r <sup>2</sup> (is less than or equal to) 0.98 in the range of 4.0 µg/g to 30.0 µg/g (w/w)

Note: CV = coefficient of variation

r<sup>2</sup> = coefficient of determination

[Ref.: Drugs Directorate Guidelines Acceptable methods, 1994]