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I

(Acts whose publication is obligatory)

COMMISSION REGULATION (EEC) No 3354/92

of 20 November 1992

fixing the import levies on cereals and on wheat or rye flour, groats and meal

THE COMMISSION OF THE EUROPEAN COMMUNITIES,
Having regard to the Treaty establishing the European Economic Community,

Having regard to the Act of Accession of Spain and Portugal,

Having regard to Council Regulation (EEC) No 2727/75 of 29 October 1975 on the common organization of the market in cereals⁽¹⁾, as last amended by Regulation (EEC) No 1738/92⁽²⁾, and in particular Article 13 (5) thereof,

Having regard to Council Regulation (EEC) No 1676/85 of 11 June 1985 on the value of the unit of account and the exchange rates to be applied for the purposes of the common agricultural policy⁽³⁾, as last amended by Regulation (EEC) No 2205/90⁽⁴⁾, and in particular Article 3 thereof,

Whereas the import levies on cereals, wheat and rye flour, and wheat groats and meal were fixed by Commission Regulation (EEC) No 1820/92⁽⁵⁾ and subsequent amending Regulations;

Whereas, if the levy system is to operate normally, levies should be calculated on the following basis:

- in the case of currencies which are maintained in relation to each other at any given moment within a band of 2,25 %, a rate of exchange based on their central rate, multiplied by the corrective factor provided for in

the last paragraph of Article 3 (1) of Regulation (EEC) No 1676/85,

- for the other currencies, an exchange rate based on an average of the ecu rates published in the *Official Journal of the European Communities*, C series, over a period to be determined, multiplied by the coefficient referred to in the preceding indent;

Whereas these exchange rates being those recorded on 19 November 1992;

Whereas the aforesaid corrective factor affects the entire calculation basis for the levies, including the equivalence coefficients;

Whereas it follows from applying the detailed rules contained in Regulation (EEC) No 1820/92 to today's offer prices and quotations known to the Commission that the levies at present in force should be altered to the amounts set out in the Annex hereto,

HAS ADOPTED THIS REGULATION:

Article 1

The import levies to be charged on products listed in Article 1 (a), (b) and (c) of Regulation (EEC) No 2727/75 shall be as set out in the Annex hereto.

Article 2

This Regulation shall enter into force on 21 November 1992.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 20 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 281, 1. 11. 1975, p. 1.

⁽²⁾ OJ No L 180, 1. 7. 1992, p. 1.

⁽³⁾ OJ No L 164, 24. 6. 1985, p. 1.

⁽⁴⁾ OJ No L 201, 31. 7. 1990, p. 9.

⁽⁵⁾ OJ No L 185, 4. 7. 1992, p. 1.

ANNEX

to the Commission Regulation of 20 November 1992 fixing the import levies on cereals
and on wheat or rye flour, groats and meal

CN code	Levy (°) (ECU/tonne)
0709 90 60	130,74 (°) (°)
0712 90 19	130,74 (°) (°)
1001 10 10	165,71 (°) (°) (°)
1001 10 90	165,71 (°) (°) (°)
1001 90 91	127,83
1001 90 99	127,83 (°)
1002 00 00	153,94 (°)
1003 00 10	121,85
1003 00 90	121,85 (°)
1004 00 10	111,85
1004 00 90	111,85
1005 10 90	130,74 (°) (°)
1005 90 00	130,74 (°) (°)
1007 00 90	137,25 (°)
1008 10 00	41,43 (°)
1008 20 00	106,55 (°)
1008 30 00	40,49 (°)
1008 90 10	(°)
1008 90 90	40,49
1101 00 00	191,80 (°) (°)
1102 10 00	228,20 (°)
1103 11 10	269,46 (°) (°)
1103 11 90	206,49 (°)

(°) Where durum wheat originating in Morocco is transported directly from that country to the Community, the levy is reduced by ECU 0,60/tonne.

(°) In accordance with Regulation (EEC) No 715/90 the levies are not applied to products imported directly into the French overseas departments, originating in the African, Caribbean and Pacific States.

(°) Where maize originating in the ACP is imported into the Community the levy is reduced by ECU 1,81/tonne.

(°) Where millet and sorghum originating in the ACP is imported into the Community the levy is applied in accordance with Regulation (EEC) No 715/90.

(°) Where durum wheat and canary seed produced in Turkey are transported directly from that country to the Community, the levy is reduced by ECU 0,60/tonne.

(°) The import levy charged on rye produced in Turkey and transported directly from that country to the Community is laid down in Council Regulation (EEC) No 1180/77 and Commission Regulation (EEC) No 2622/71.

(°) The levy applicable to rye shall be charged on imports of the product falling within CN code 1008 90 10 (triticale).

(°) On importation into Portugal the levy is increased by the amount specified in Article 2 (2) of Regulation (EEC) No 3808/90.

(°) No levy applies to OCT originating products according to Article 101 (1) of Decision 91/482/EEC, except if paragraph 4 of the same Article applies.

(°) An amount equal to the amount fixed by Regulation (EEC) No 1825/91 is to be levied in accordance with Article 101 (4) of Decision 91/482/EEC.

(°) Products falling within this code, imported from Poland, Czechoslovakia or Hungary under the Interim Agreements concluded between those countries and the Community, and in respect of which EUR.1 certificates issued in accordance with Regulation (EEC) No 585/92 have been presented, are subject to the levies set out in the Annex to that Regulation.

COMMISSION REGULATION (EEC) No 3355/92

of 20 November 1992

fixing the premiums to be added to the import levies on cereals, flour and malt

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 2727/75 of 29 October 1975 on the common organization of the market in cereals ⁽¹⁾, as last amended by Regulation (EEC) No 1738/92 ⁽²⁾, and in particular Article 15 (6) thereof,Having regard to Council Regulation (EEC) No 1676/85 of 11 June 1985 on the value of the unit of account and the exchange rates to be applied for the purposes of the common agricultural policy ⁽³⁾, as last amended by Regulation (EEC) No 2205/90 ⁽⁴⁾, and in particular Article 3 thereof,Whereas the premiums to be added to the levies on cereals and malt were fixed by Commission Regulation (EEC) No 1821/92 ⁽⁵⁾ and subsequent amending Regulations;

Whereas, if the levy system is to operate normally, levies should be calculated on the following basis:

- in the case of currencies which are maintained in relation to each other at any given moment within a band of 2,25 %, a rate of exchange based on their central rate, multiplied by the corrective factor provided for in the last paragraph of Article 3 (1) of Regulation (EEC) No 1676/85,

- for the other currencies, an exchange rate based on an average of the ecu rates published in the *Official Journal of the European Communities*, C series, over a period to be determined, multiplied by the coefficient referred to in the preceding indent;

Whereas these exchange rates being those recorded on 19 November 1992;

Whereas, on the basis of today's cif prices and cif forward delivery prices, the premiums at present in force, which are to be added to the levies, should be altered to the amounts set out in the Annex hereto,

HAS ADOPTED THIS REGULATION:

Article 1

The premiums referred to in Article 15 of Regulation (EEC) No 2727/75 to be added to the import levies fixed in advance in respect of cereals and malt coming from third countries shall be as set out in the Annex hereto.

Article 2

This Regulation shall enter into force on 21 November 1992.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 20 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 281, 1. 11. 1975, p. 1.

⁽²⁾ OJ No L 180, 1. 7. 1992, p. 1.

⁽³⁾ OJ No L 164, 24. 6. 1985, p. 1.

⁽⁴⁾ OJ No L 201, 31. 7. 1990, p. 9.

⁽⁵⁾ OJ No L 185, 4. 7. 1992, p. 4.

ANNEX

to the Commission Regulation of 20 November 1992 fixing the premiums to be added to the import levies on cereals, flour and malt

A. Cereals and flour

(ECU/tonne)

CN code	Current 11	1st period 12	2nd period 1	3rd period 2
0709 90 60	0	0	0	0
0712 90 19	0	0	0	0
1001 10 10	0	0	0	0
1001 10 90	0	0	0	0
1001 90 91	0	16,41	16,41	16,41
1001 90 99	0	16,41	16,41	16,41
1002 00 00	0	0	0	0
1003 00 10	0	0	0	0
1003 00 90	0	0	0	0
1004 00 10	0	0,24	0,24	0,24
1004 00 90	0	0,24	0,24	0,24
1005 10 90	0	0	0	0
1005 90 00	0	0	0	0
1007 00 90	0	0	0	0
1008 10 00	0	0	0	0
1008 20 00	0	0	0	0
1008 30 00	0	0	0	0
1008 90 90	0	0	0	0
1101 00 00	0	22,97	22,97	22,97

B. Malt

(ECU/tonne)

CN code	Current 11	1st period 12	2nd period 1	3rd period 2	4th period 3
1107 10 11	0	29,21	29,21	29,21	29,21
1107 10 19	0	21,83	21,83	21,83	21,83
1107 10 91	0	0	0	0	0
1107 10 99	0	0	0	0	0
1107 20 00	0	0	0	0	0

COMMISSION REGULATION (EEC) No 3356/92
of 19 November 1992
on the supply of milk products as food aid

THE COMMISSION OF THE EUROPEAN COMMUNITIES,
Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 3972/86 of 22 December 1986 on food-aid policy and food-aid management ⁽¹⁾, as last amended by Regulation (EEC) No 1930/90 ⁽²⁾, and in particular Article 6 (1) (c) thereof,

Whereas Council Regulation (EEC) No 1420/87 of 21 May 1987 laying down implementing rules for Regulation (EEC) No 3972/86 on food-aid policy and food-aid management ⁽³⁾ lays down the list of countries and organizations eligible for food-aid operations and specifies the general criteria on the transport of food aid beyond the fob stage;

Whereas following the taking of a number of decisions on the allocation of food aid the Commission has allocated to certain recipients 2 936 tonnes of skimmed-milk powder;

Whereas it is necessary to provide for the carrying-out of this measure in accordance with the rules laid down by Commission Regulation (EEC) No 2200/87 of 8 July 1987 laying down general rules for the mobilization in the Community of products to be supplied as Community food aid ⁽⁴⁾, as amended by Regulation (EEC) No 790/91 ⁽⁵⁾; whereas it is necessary to specify the time limits and conditions of supply and the procedure to be followed to determine the resultant costs;

Whereas, notably for logistical reasons, certain supplies are not awarded within the first and second deadlines for submissions of tenders; whereas, in order to avoid re-publication of the notice of invitation to tender, a third deadline for submission of tenders should be opened,

HAS ADOPTED THIS REGULATION:

Article 1

Milk products shall be mobilized in the Community, as Community food aid, for supply to the recipients listed in the Annexes in accordance with Regulation (EEC) No 2200/87 and under the conditions set out in the Annexes. Supplies shall be awarded by the tendering procedure.

The successful tenderer is deemed to have noted and accepted all the general and specific conditions applicable. Any other condition or reservation included in his tender is deemed unwritten.

Article 2

This Regulation shall enter into force on the day following its publication in the *Official Journal of the European Communities*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 19 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 370, 30. 12. 1986, p. 1.

⁽²⁾ OJ No L 174, 7. 7. 1990, p. 6.

⁽³⁾ OJ No L 136, 26. 5. 1987, p. 1.

⁽⁴⁾ OJ No L 204, 25. 7. 1987, p. 1.

⁽⁵⁾ OJ No L 81, 28. 3. 1991, p. 108.

ANNEX I

LOTS A

1. **Operation Nos** ⁽¹⁾: 826/92 (A1); 118/92 (A2)
2. **Programme**: 1992
3. **Recipient** ⁽²⁾: Euronaid, PO Box 79, NL-3240 AB Oegstgeest, Nederland; tel. (31 71) 15 91 56/59; fax 15 52 01; telex 30223 NL CEMEC
4. **Representative of the recipient** ⁽³⁾: see OJ No C 103, 16. 4. 1987
5. **Place or countries of destination**: Haiti (A1), Madagascar (A2)
6. **Product to be mobilized**: vitaminized skimmed-milk powder
7. **Characteristics and quality of the goods** ⁽⁴⁾ ⁽⁵⁾:
(see OJ No C 114, 29. 4. 1991, p. 3 (under I.B.1))
8. **Total quantity**: 185 tonnes
9. **Number of lots**: one (A1: 170 tonnes; A2: 15 tonnes)
10. **Packaging and marking** ⁽⁷⁾ ⁽⁸⁾:
see OJ No C 114, 29. 4. 1991, p. 4 (under I.A.2.3, I.B.2 and I.B.3)
markings in French
supplementary markings: A1: 'Protos / 921502 / Port-au-Prince'
A2: 'Appel Détresse / 926802 / Antananarivo via Toamasina'
11. **Method of mobilization**: the Community market
The vitaminized skimmed-milk powder must be manufactured after the award of the tender
12. **Stage of supply**: free at port of shipment
13. **Port of shipment**: —
14. **Port of landing specified by the recipient**: —
15. **Port of landing**: —
16. **Address of the warehouse and, if appropriate, port of landing**: —
17. **Period for making the goods available at the port of shipment**: 4 — 24. 1. 1993
18. **Deadline for the supply**: —
19. **Procedure for determining the costs of supply**: Invitation to tender
20. **Date of expiry of the period allowed for submission of tenders**: 12 noon (Brussels time) on 7. 12. 1992
21. **A. In the case of a second invitation to tender**:
 - (a) deadline for the submission of tenders: 12 noon (Brussels time) on 21. 12. 1992
 - (b) period for making the goods available at the port of shipment: 18. 01 — 7. 2. 1993
 - (c) deadline for the supply: —**B. In the case of a third invitation to tender**:
 - (a) deadline for the submission of tenders: 12 noon (Brussels time) on 4. 1. 1993
 - (b) period for making the goods available at the port of shipment: 1 — 21. 2. 1993
 - (c) deadline for the supply: —
22. **Amount of tendering security**: ECU 20 per tonne
23. **Amount of delivery security**: 10 % of the tender in ecus
24. **Address for submission of tenders and tendering securities** ⁽¹⁾: Bureau de l'aide alimentaire, à l'attention de Monsieur N. Arend, bâtiment Loi 120, bureau 7/46, rue de la Loi 200, B-1049 Bruxelles; (telex 22037 / 25670 AGREC B; telefax: (32-2) 296 20 05 / 295 01 32 / 296 10 97 / 295 01 30 c/ 296 33 04)
25. **Refund payable on application by the successful tenderer** ⁽⁴⁾: refund applicable 25. 11. 1992, fixed by Commission Regulation (EEC) No 3290/92 (OJ No L 327, 13. 11. 1992, p. 34)

LOTS B, C, D, E and F

1. **Operation Nos** (1): 1125/92 (Lot B), 1137/92 (Lot C), 1126/92 (Lot D), 1138/92 (Lot E), 1139/92 (Lot F)
2. **Programme**: 1992
3. **Recipient** (2): UNRWA — Supply Division — Vienna International Centre, PO Box 700, A-1400 Vienna, Austria telex: 135310 A — fax (1) 230 75 29
4. **Representative of the recipient**: UNRWA Field Supply and Transport Officer,
 - Ashdod: West Bank, PO Box 19149 Jerusalem, Israel; (tel. (972-3) 82 80 93; telefax 81 65 64; telex (0606) 26194 IL UNRWA)
 - Latakia: PO Box 4313, Damascus, Syrian Arab Republic; (tel. (963-11) 66 02 17; telex (0492) 412006 SY UNRWA)
 - Beirut: PO Box 947, Beirut, Lebanon; (tel. (961-9) 81 00 12; telefax 871-145 02 32; telex (0494) 21430 LE UNRWA)
 - Amman: PO Box 484, Amman, Jordan; (tel. (962-6) 77 17 41; telefax: 68 54 76; telex (0493) 23402 JO UNRWA)
5. **Place or country of destination** (3): Lot B: Israel; Lot C: Syria; Lots D and E: Lebanon; Lot F: Jordan
6. **Product to be mobilized**: Vitaminized skimmed-milk powder
7. **Characteristics and quality of the goods** (3) (4): (See OJ No C 114, 29. 4. 1991, p. 3-4, I.B.1)
8. **Total quantity**: 506 tonnes
9. **Number of lots**: five (Lot B: 108 tonnes; Lot C: 108 tonnes; Lot D: 20 tonnes; Lot E: 167 tonnes; Lot F: 165 tonnes)
10. **Packaging and marking** (7): 1 kg sachets
 - See OJ No C 114, 29. 4. 1991, p. 4. (I.B.2, I.B.3 and I.A.2.1)
 - Markings in English
 - Supplementary markings:
 - Lots B, C, D and E: 'UNRWA'
 - Lot F: 'UNRWA — Date of expiry ...' (date of manufacture plus one year)
11. **Method of mobilization**: Community market
 - The skimmed-milk powder must be manufactured and the vitamins added after the award of the tender
12. **Stage of supply**: Lots B, C, D and E: free at port of landing — landed; Lot F: free at destination
13. **Port of shipment**: —
14. **Port of landing specified by the recipient**: —
15. **Port of landing**: Lot B: Ashdod; Lot C: Latakia; Lots D and E: Beirut
16. **Address of the warehouse and, if appropriate, port of landing**: Lot F: UNRWA warehouses, Amman, Jordan
17. **Period for making the goods available at the port of shipment where the supply is awarded at the port of shipment stage**: 4 — 17. 1. 1993
18. **Deadline for the supply**: 7. 2. 1993
19. **Procedure for determining the costs of supply**: invitation to tender
20. **Date of expiry of the period allowed for submission of tenders**: 12 noon on 7. 12. 1992

21. A. In the case of a second invitation to tender :

- (a) deadline for the submission of tenders : 12 noon (Brussels time) on 21. 12. 1992
- (b) period for making the goods available at the port of shipment in case of award of tender at port of shipment stage : 18 — 31. 1. 1993
- (c) deadline for the supply : 21. 2. 1993

B. In the case of a third invitation to tender :

- (a) deadline for the submission of tenders : 12 noon (Brussels time) on 4. 1. 1993
- (b) period for making the goods available at the port of shipment in case of award of tender at port of shipment stage : 1 — 14. 2. 1993
- (c) deadline for the supply : 7. 3. 1993

22. Amount of tendering security : ECU 20 per tonne**23. Amount of delivery security : 10 % of the tender in ecus****24. Address for submission of tenders and tendering securities⁽¹⁾ :** Bureau de l'aide alimentaire, à l'attention de Monsieur N. Arend, bâtiment Loi 120, bureau 7/46, rue de la Loi, 200, B-1049 Bruxelles (telex 22037 / 25670 AGREC B ; telefax : (32 2) 296 20 05 / 295 01 32 / 296 10 97 / 295 01 30 / 296 33 04)**25. Refund payable on application by the successful tenderer^(*) :** Refund applicable 25. 11. 1992 fixed by Commission Regulation (EEC) No 3290/92 (OJ No L 327, 13. 11. 1992, p. 34)

3. Incubation of cell cultures

Inoculated cell cultures are incubated at 15 °C for seven days. If the colour of the cell culture medium changes from red to yellow indicating medium acidification, pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to ensure cell susceptibility to virus infection.

Titration every six months of frozen stocks of VHSV and IHNV is performed to verify the susceptibility of the cell cultures to infection.

4. Microscopy

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 × magnification. If obvious CPE is observed, virus identification procedures according to section IV have to be initiated immediately.

At the same time appropriate steps are taken to suspend the approved status of the production unit from where the virus positive sample originated as well as from any production units situated downstream.

Suspension of approved status has to be maintained until laboratory tests have proved the virus in question not to be VHSV or IHNV. A maximum of four weeks are allowed for identification of the virus, including the time needed for examination in reference laboratories.

5. Subcultivation

If no CPE has developed after incubation for seven days, subcultivation is performed to fresh cell cultures utilizing a cell area similar to that of the primary culture.

Aliquots of medium from all cultures/wells constituting the primary culture are pooled according to cell line following one freeze-thaw cycle of the cultures and 0,5 ml of the medium mixed with equal parts of antiserum to IPN as described in Section II.3. and the mixture incubated at 15 °C for 60 minutes. The mixture is then inoculated into homologous cell cultures at dilutions 1 : 1 and 1 : 100 as described in section III.2. The inoculation may be preceded by preincubation of the dilutions with divalent antiserum to IPN virus at appropriate dilution.

The inoculated cultures are then incubated for seven days at 15 °C with observation as in Section III.4.

IV. Virus identification

1. Neutralization

If evidence of CPE has been observed in a cell culture, medium is collected, cells removed by low speed centrifugation or membrane filtration (0.45 µm) and medium then diluted 1 : 100 and 1 : 10 000 in cell culture medium.

Aliquots of the dilutions are mixed and incubated for 60 minutes at 15 °C with equal parts of the following reagents separately:

— group specific antibody to VHSV (Egtved virus)	1 : 50 (*)
— group specific antibody to infectious hematopoietic necrosis virus (IHNV)	1 : 50 (*)
— specific divalent antiserum to infectious pancreatic necrosis virus (IPNV) (reference strains Sp and Ab)	1 : 50 (*)
— medium	1 : 1

(*) or as specified by the reference laboratory with regard to the possible cytotoxicity of the antisera.

The reagents used have to be of reference quality with regard to titre and specificity.

From each virus-serum mixture at least two cell cultures are inoculated with 50 UI each and then incubated at 15 °C. Development of CPE is checked as described in Section III.4.

If the test has not allowed safe identification of the virus within one week, one of the following steps has to be taken:

- transfer of the virus to a national fish virus reference laboratory for immediate identification;
- application of immunofluorescence (IFAT) (Section IV.2), enzyme-linked immunosorbent assay (ELISA) (Section IV.3) or other virus identification techniques with reagents of reference quality.

LOT H

1. **Operation No** (1): 1235/92
2. **Programme**: 1992
3. **Recipient** (2): World Food Programme, Via Cristoforo Colombo 426, I-00145 Roma (telex 626675 i wfp)
4. **Representative of the recipient**: see OJ No C 103, 16. 4. 1987
5. **Place or country of destination**: Cuba
6. **Product to be mobilized**: skimmed-milk powder
7. **Characteristics and quality of the goods** (3) (4): see OJ No C 114, 29. 4. 1991, p. 3-4, I.A. 1.
8. **Total quantity**: 875 tonnes
9. **Number of lots**: one
10. **Packaging and marking**: see OJ No C 114, 29. 4. 1991, p. 4 (I.A. 2. 3. and I.A. 3.)
Markings in English
Supplementary markings:
See Annex II
11. **Method of mobilization of product**: Community market
The skimmed-milk powder must be manufactured after the award of the tender
12. **Stage of supply**: free at port of shipment
13. **Port of shipment**: —
14. **Port of landing specified by the recipient**: —
15. **Port of landing**: —
16. **Address of the warehouse and, if appropriate, port of landing**: —
17. **Period for making the goods available at the port of shipment**: 11. 1. — 7. 2. 1993
18. **Deadline for the supply**: —
19. **Procedure for determining the costs of supply**: invitation to tender
20. **In the case of an invitation to tender, date of expiry of the period allowed for submission of tenders**: 12 noon (Brussels time) on 7. 12. 1992
21. **A. In the case of a second invitation to tender**:
 - (a) deadline for the submission of tenders: 12 noon (Brussels time) on 21. 12. 1992
 - (b) period for making the goods available at the port of shipment: 25. 1. — 21. 2. 1993
 - (c) deadline for the supply: —**B. In the case of a third invitation to tender**:
 - (a) deadline for the submission of tenders: 12 noon (Brussels time) on 4. 1. 1993
 - (b) period for making the goods available at the port of shipment: 8. 2. — 7. 3. 1993
 - (c) deadline for the supply: —
22. **Amount of the tendering security**: ECU 20 per tonne
23. **Amount of the delivery security**: 10 % of the tender in ecus
24. **Address for submission of tenders and tendering securities** (5): Bureau de l'aide alimentaire, à l'attention Mr N. Arend, bâtiment Loi 120, bureau 7/46, rue de la Loi 200, B-1049 Bruxelles; (telex 22037 / 25670 AGREC B; telefax: (32-2) 296 20 05 / 295 01 32 / 296 10 97 / 295 01 30 / 296 33 04)
25. **Refund payable on request by the successful tenderer** (6): refund applicable on 25. 11. 1992, fixed by Commission Regulation (EEC) No 3290/92 (OJ No L 327, 13. 11. 1992, p. 34)

Notes:

- (¹) The operation number should be mentioned in all correspondence.
- (²) The successful tenderer shall contact the recipient as soon as possible to establish which consignment documents are required.
- (³) The successful tenderer shall deliver to the beneficiary a certificate from an official entity certifying that for the product to be delivered the standards applicable, relative to nuclear radiation, in the Member State concerned, have not been exceeded.

The radioactivity certificate must indicate the caesium-134 and -137 and iodine-131 levels.

- (⁴) Commission Regulation (EEC) No 2330/87 (OJ No L 210, 1. 8. 1987, p. 56), as last amended by Regulation (EEC) No 2226/89 (OJ No L 214, 25. 7. 1989, p. 10), is applicable as regards the export refund and, where appropriate, the monetary and accession compensatory amounts, the representative rate and the monetary coefficient. The date referred to in Article 2 of the abovementioned Regulation is that referred to in point 25 of this Annex.
- (⁵) Commission delegation to be contacted by the successful tenderer: see OJ No C 114, 29. 4. 1991, p. 33.
- (⁶) The successful tenderer shall supply to the beneficiary or its representative, on delivery, the following documents:
 - health certificate,
 - certificate of origin.

Veterinary certificate issued by an official entity stating that the product was processed with pasteurized milk, coming from healthy animals, processed under excellent sanitary conditions which are supervised by qualified technical personnel and that the area of production of raw milk had not registered foot-and-mouth disease nor any other notifiable infectious/contagious disease during the 90 days prior to the processing.

Lot C: The health certificate and the certificate of origin must be signed and stamped by a Syrian Consulate, including a statement that consular fees and charges have been paid.

- (⁷) Shipment to take place in 20-foot containers. Lots B, C, D and E: The contracted shipping terms shall be considered full liner terms (liner in/liner out) free Ashdod/Beirut/Latakia, container yard and are understood to cover 15 days — Saturdays, Sundays and official public and religious holidays excluded — free of container detention charges at the port of discharge taken from the day/time of the arrival of the vessel. The 15 days free of container detention charges should be clearly marked on the bill of lading. Bona fide detention charges levied in respect of container detention(s) in excess of the said 15 days as detailed above will be borne by UNRWA. UNRWA shall not pay/not be charged any container deposit fees.

After take over of the goods at the delivery stage, the recipient will bear all costs of shifting the containers for destuffing outside the port area and of returning them to the container yard.

Ashdod: consignment to be stowed in 20-foot containers not more than 17 tonnes each, net, not more than 50 containers being shipped per week on any vessel.

- (⁸) The supplier should send a duplicate of the original invoice to:

MM De Keyzer & Schütz BV,
Postbus 1438,
Blaak 16,
NL-3000 BK Rotterdam.
- (⁹) Shipment to take place in 20-foot containers, condition FCL/LCL. The supplier shall be responsible for the cost of making the containers available in the stack position at the container terminal at the port of shipment.

The successful tenderer has to submit to the recipient's agent a complete packaging list of each container, specifying the number of bags belonging to each shipping number as specified in the invitation to tender.

The successful tenderer has to seal each container with a numbered locktainer, the number of which is to be provided to the beneficiary's forwarder.
- (¹⁰) G3: To be delivered on standard pallets — 40 bags each pallet — wrapped in plastic shrink cover.

ANEXO II — BILAG II — ANHANG II — ΠΑΡΑΡΤΗΜΑ II — ANNEX II — ANNEXE II — ALLEGATO II — BIJLAGE II — ANEXO II

Lote	Cantidad total (en toneladas)	Cantidades parciales (en toneladas)	Acción n°	Inscripciones complementarias
Parti	Totalmængde (i tons)	Delmængde (i tons)	Aktion nr.	Yderligere påskrifter
Partie	Gesamtmenge (in Tonnen)	Teilmengen (in Tonnen)	Maßnahme Nr.	Ergänzende Aufschriften
Παρτίδα	Συνολική ποσότητα (σε τόνους)	Μερικές ποσότητες (σε τόνους)	Δράση αριθ.	Συμπληρωματικές ενδείξεις
Lot	Total quantity (in tonnes)	Partial quantities (in tonnes)	Operation No	Supplementary markings
Lot	Quantité totale (en tonnes)	Quantités partielles (en tonnes)	Action n°	Marquage complémentaire
Lotto	Quantità totale (in tonnellate)	Quantitativi parziali (in tonnellate)	Azione n.	Iscrizioni supplementari
Partij	Totale hoeveelheid (in ton)	Deelhoeveelheden (in ton)	Maatregel nr.	Bijkomende vermeldingen
Lote	Quantidade total (em toneladas)	Quantidades parciais (em toneladas)	Acção n°	Inscrições complementares
G	1 370	G1 : 50	1226/92	WFP / 0475600 / Zambia via Durban
		G2 : 78	1227/92	WFP / 0475600 / Zambia via Durban
		G3 : 65	1228/92	WFP / 0373400 / Phuntsholing, Bhutan via Calcutta
		G4 : 150	1229/92	WFP / 0225404 / Bissau
		G5 : 45	1230/92	WFP / 0260202 / Malabo
		G6 : 369	1231/92	WFP / 0228803 / Tangier
		G7 : 288	1232/92	WFP / 0228803 / Tangier
		G8 : 35	1233/92	WFP / 0228803 / Casablanca
		G9 : 290	1234/92	WFP / 0450001 / Namibia via Walvisbay
H	875		1235/92	WFP / Cuba / 0439100 / La Habana

COMMISSION REGULATION (EEC) No 3357/92
of 20 November 1992
fixing the import levies on white sugar and raw sugar

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 1785/81 of 30 June 1981 on the common organization of the markets in the sugar sector ⁽¹⁾, as last amended by Regulation (EEC) No 61/92 ⁽²⁾, and in particular Article 16 (8) thereof,

Whereas the import levies on white sugar and raw sugar were fixed by Commission Regulation (EEC) No 1813/92 ⁽³⁾, as last amended by Regulation (EEC) No 3348/92 ⁽⁴⁾;

Whereas it follows from applying the detailed rules contained in Commission Regulation (EEC) No 1813/92 to the information known to the Commission that the levies at present in force should be altered to the amounts set out in the Annex hereto;

Whereas, if the levy system is to operate normally, levies should be calculated on the following basis:

- in the case of currencies which are maintained in relation to each other at any given moment within a band of 2,25 %, a rate of exchange based on their central rate, multiplied by the corrective factor provided for in

the last subparagraph of Article 3 (1) of Council Regulation (EEC) No 1676/85 ⁽⁵⁾, as last amended by Regulation (EEC) No 2205/90 ⁽⁶⁾,

- for the other currencies, an exchange rate based on an average of the ecu rates published in the *Official Journal of the European Communities*, C series, over a period to be determined, multiplied by the coefficient referred to in the preceding indent;

Whereas these exchange rates being those recorded on 19 November 1992,

HAS ADOPTED THIS REGULATION:

Article 1

The import levies referred to in Article 16 (1) of Regulation (EEC) No 1785/81 shall be, in respect of white sugar and standard quality raw sugar, as set out in the Annex hereto.

Article 2

This Regulation shall enter into force on 21 November 1992.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 20 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 177, 1. 7. 1981, p. 4.

⁽²⁾ OJ No L 6, 11. 1. 1992, p. 19.

⁽³⁾ OJ No L 183, 3. 7. 1992, p. 18.

⁽⁴⁾ OJ No L 336, 20. 11. 1992, p. 22.

⁽⁵⁾ OJ No L 164, 24. 6. 1985, p. 1.

⁽⁶⁾ OJ No L 201, 31. 7. 1990, p. 9.

ANNEX

to the Commission Regulation of 20 November 1992 fixing the import levies on white sugar and raw sugar

(ECU/100 kg)

CN code	Levy ⁽¹⁾
1701 11 10	39,16 ⁽¹⁾
1701 11 90	39,16 ⁽¹⁾
1701 12 10	39,16 ⁽¹⁾
1701 12 90	39,16 ⁽¹⁾
1701 91 00	46,02
1701 99 10	46,02
1701 99 90	46,02 ⁽²⁾

⁽¹⁾ The levy applicable is calculated in accordance with the provisions of Article 2 or 3 of Commission Regulation (EEC) No 837/68.

⁽²⁾ In accordance with Article 16 (2) of Regulation (EEC) No 1785/81 this amount is also applicable to sugar obtained from white and raw sugar containing added substances other than flavouring or colouring matter.

⁽³⁾ No import levy applies to OCT originating products according to Article 101 (1) of Decision 91/482/EEC. However, an amount equal to the amount fixed by Regulation (EEC) No 1870/91 B to be levied in accordance with Article 101 (4) of the abovementioned Decision.

COMMISSION REGULATION (EEC) No 3358/92

of 20 November 1992

altering the export refunds on cereals and on wheat or rye flour, groats and meal

THE COMMISSION OF THE EUROPEAN COMMUNITIES,
Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 2727/75 of 29 October 1975 on the common organization of the market in cereals ⁽¹⁾, as last amended by Regulation (EEC) No 1738/92 ⁽²⁾, and in particular the fifth subparagraph of Article 16(2) thereof,

Whereas the export refunds on cereals and on wheat or rye flour, groats and meal were fixed by Commission Regulation (EEC) No 3350/92 ⁽³⁾;

Whereas export possibilities exist for a quantity of 50 000 tonnes of rye flour of certain destinations; whereas the procedure laid down in Article 9 (4) of Commission Regulation (EEC) No 891/89 ⁽⁴⁾, as last amended by Regulation (EEC) No 2804/92 ⁽⁵⁾, should be used; whereas account should be taken of this when the refunds are fixed;

Whereas it follows from applying the detailed rules contained in Commission Regulation (EEC) No 3350/92

to the information known to the Commission that the export refunds at present in force should be altered to the amounts set out in the Annex hereto,

HAS ADOPTED THIS REGULATION:

Article 1

The export refunds on the products listed in Article 1 (a), (b) and (c) of Regulation (EEC) No 2727/75, exported in the natural state, as fixed in the Annex to Regulation (EEC) No 3350/92 are hereby altered as shown in the Annex to this Regulation in respect of the products set out therein.

Article 2

This Regulation shall enter into force on 21 November 1992.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 20 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 281, 1. 11. 1975, p. 1.

⁽²⁾ OJ No L 180, 1. 7. 1992, p. 1.

⁽³⁾ OJ No L 336, 20. 11. 1992, p. 25.

⁽⁴⁾ OJ No L 94, 7. 4. 1989, p. 13.

⁽⁵⁾ OJ No L 282, 26. 9. 1992, p. 40.

ANNEX

to the Commission Regulation of 20 November 1992 amending export refunds on cereals
and on wheat or rye flour, groats and meal

(ECU/tonne)		
Product code	Destination (1)	Amount of refund (2)
0709 90 60 000	—	—
0712 90 19 000	—	—
1001 10 10 000	—	—
1001 10 90 000	04	50,00
	02	20,00
1001 90 91 000	01	0
1001 90 99 000	04	63,00
	05	21,00
	02	20,00
1002 00 00 000	03	21,00
	02	20,00
1003 00 10 000	06	65,00
	02	0
1003 00 90 000	04	65,00
	02	20,00
1004 00 10 000	—	—
1004 00 90 000	—	—
1005 10 90 000	—	—
1005 90 00 000	04	80,00
	02	0
1007 00 90 000	—	—
1008 20 00 000	—	—
1101 00 00 100	01	100,00
1101 00 00 130	01	94,00
1101 00 00 150	01	87,00
1101 00 00 170	01	80,00
1101 00 00 180	01	75,00
1101 00 00 190	—	—
1101 00 00 900	—	—
1102 10 00 500	01	123,50 (3)
1102 10 00 700	—	—
1102 10 00 900	—	—
1103 11 10 200	01	140,00
1103 11 10 400	01	120,00
1103 11 10 900	01	0
1103 11 90 200	01	100,00
1103 11 90 800	—	—

(¹) The destinations are identified as follows :

- 01 All third countries,
- 02 Other third countries,
- 03 Switzerland, Austria and Liechtenstein,
- 04 Switzerland, Austria, Liechtenstein, Ceuta and Melilla,
- 05 Poland,
- 06 zone II c).

(²) Refunds for exports to the Republics of Serbia and Montenegro may only be granted for humanitarian aid supplied by charitable organizations fulfilling the conditions laid down in Article 2 (a) and Article 3 of Council Regulation (EEC) No 1432/92.

(³) Refund fixed under the procedure laid down in Article 9 (4) of amended Regulation (EEC) No 891/89 in respect of a quantity of 50 000 tonnes of rye flour destined for all third countries.

NB: The zones are those defined in Commission Regulation (EEC) No 2145/92 (OJ No L 214, 30. 7. 1992, p. 20).

II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DECISION

of 19 November 1992

laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases

(92/532/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 91/67/EEC of 28 January 1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products⁽¹⁾, and in particular Article 15 thereof,

Whereas, in accordance with Article 15 of Directive 91/67/EEC sampling plans and diagnostic methods to be applied for the detection and confirmation of diseases in aquaculture animals shall be established;

Whereas the Scientific Veterinary Committee, established by Commission Decision 81/651/EEC⁽²⁾ has been consulted;

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

The sampling plans and diagnostic methods for the detection and confirmation of infectious Haematopoietic Necrosis (IHN) and Viral haemorrhagic Septicaemia (VHS) are laid down in the Annex.

Article 2

This Decision is addressed to the Member States.

Done at Brussels, 19 November 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

⁽¹⁾ OJ No L 46, 19. 2. 1991, p. 1.

⁽²⁾ OJ No L 233, 19. 8. 1981, p. 32.

ANNEX

PART I

SAMPLING AND TESTING PROCEDURES FOR VHS AND IHN MONITORING

I. Sampling

1. *Sampling time*

Farms are inspected clinically at least twice per year during the period October to June or whenever the water temperature is below 14 °C. Intervals between inspections must be at least four months. All production units (ponds, tanks, aquaria, netcages, etc.) are inspected for the presence of dead, weak or abnormally behaving fish. Particular attention has to be paid to the water outlet area (if feasible) where weak fish tend to accumulate because of the water current.

2. *Selection and collection of samples*

Thirty to 150 fish and/or ovarian fluid samples are collected for examination in connection with the inspections according to Table 1. If rainbow trout are present fish of that species should make up the whole sample. If rainbow trout are not present the sample has to contain fish of all other species present whenever these species are susceptible to VHS and/or IHN as listed in Annex A of Council Directive 91/67/EEC concerning the animal health conditions governing the placing on the market of aquaculture animals and products. The species have to be equally represented in the sample. During the initial four-year control period which precedes achievement of approved status the sample size is 150 in order to ensure detection at a 95 % confidence level of virus carriers at a carrier prevalence of 2 %. During the subsequent years (maintenance of approved status) the sample size can be reduced to 30 to ensure detection at a 95 % confidence level of virus at a prevalence of 10 %.

In farms which have a documented history of freedom from VHS and IHN (based on a regular official health inspection programme) the small sample size can be used also during the initial four-year control.

If more than one water source is utilized for fish production, fish representing all water sources may be included in the 150 or 30 fish-sample. If weak, abnormally behaving or freshly dead (not decomposed) fish are present, these must primarily be included in the sample. If such fish are not present the sample must be composed of normally appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are represented in the sample.

3. *Preparation and shipment of samples from fish*

Before shipment or transfer to the laboratory pieces of the organs to be examined are removed from the fish with sterile scissors and forceps and transferred to plastic tubes containing transportation medium i.e. cell culture medium with 10 % calf serum and antibiotics. The combination of 200 iu penicillin, 200 µg streptomycin, and 200 µg kanamycin per millilitre (ml) can be recommended but other antibiotics of proven efficiency may be used as well. The tissue material to be examined is spleen, anterior kidney, encephalon and, in some cases, ovarian fluid (Table 1).

Organ pieces from 5 to 10 fish (Table 1) may be collected in one tube and represent one pooled sample. The tissue in each sample should weigh a minimum of 1 gram (g) and such that the final dilution 1 : 10.

The tubes are placed in insulated containers (for instance thick-walled polystyrene boxes) together with sufficient ice of 'frost blocks' to ensure chilling of the samples to between 0 and 5 °C during transportation to the laboratory. Freezing must be avoided.

The virological examination must be started as soon as possible and not later than 48 hours after the collection of the samples. If the fish to be examined are less than 6 centimetres (cm) in length, the whole fish may be shipped to the laboratory in plastic bags chilled as mentioned above.

4. *Collection of supplementary diagnostic material*

According to agreement with the involved diagnostic laboratory, other fish tissues may be collected as well and prepared for supplementary examinations.

II. Preparation of samples for virological examination

1. *Homogenization of organs*

In the laboratory the tissue in the tubes must be completely homogenized (either by stomacher, blender or mortar and pestle) and subsequently suspended in the original transport medium. If a sample consisted of whole fish, i.e. fish less than 6 cm long, these are minced with sterile scissors after removal of the body behind the gut opening, homogenized as described above and suspended at a 1:10 ratio in transport medium.

2. *Centrifugation of homogenate*

The homogenate is centrifuged in a refrigerated centrifuge at 2 - 5 °C at 2 000 to 4 000 x g for 15 minutes and the supernatant collected for examination.

If shipment of the sample has been made in a transport medium (i.e. with exposure to antibiotics) the supernatant needs to further treatment with antibiotics.

If the sample was shipped as whole fish the homogenate following centrifugation has to be exposed to the antibiotics in the transport medium used for resuspension for either four hours at room temperature or over night at 4 °C.

The antibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary.

Immediately after the centrifugation a volume of the supernatant is mixed with equal parts of a suitably diluted divalent antiserum to IPN virus (reference strains Sp and Ab) and incubated with this for a minimum of one hour at 15 °C or a maximum of 18 hours at 4 °C. The titre of the antiserum must be at least 1/2 000 in a 50 % plaque neutralization test.

Treatment of all inocula with antiserum to IPN virus (a virus which is some parts of Europe occurs in 50 % of fish samples) aims at preventing CPE due to IPN virus from developing in inoculated cell cultures. This will reduce duration of the virological examinations as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHS or IHN.

When samples come from production units which are considered free from IPN, treatment of inocula with antiserum to IPN virus may be omitted.

III. Virological examination

1. *Cell cultures and media*

BF-2 and either EPC or FHM cells are grown at 20 to 25 °C in Eagle's MEM (or modifications thereof) with a supplement of 10 % foetal bovine serum and antibiotics in standard concentrations.

When the cells are cultivated in closed vials, the medium is buffered with bicarbonate. The medium used for cultivation of cells in open units has to be buffered with Tris-HCl (23 mM) and Na-bicarbonate (6 mM) at a pH as close as possible to 7,6, a pH which is optimal for virus multiplication.

Cell cultures to be used for inoculation with tissue material should be young (4 to 48 hours old) and actively growing (not confluent) at inoculation.

2. *Inoculation of cell cultures*

Antibiotic-treated organ suspension is inoculated into cell cultures in two dilutions, i.e. the primary dilution and, in addition, a 1:100 dilution thereof (in order to prevent homologous interference). At least two cell lines have to be inoculated (see III.1.). The ratio between inoculum size and volume of cell culture medium should be about 1:10.

For each dilution and each cell line a minimum of about 2 cm² cell area, corresponding to one well in a 24-well cell culture tray, has to be utilized. Use of cell culture trays is recommended, but other units of similar or bigger growth area are acceptable as well.

3. *Incubation of cell cultures*

Inoculated cell cultures are incubated at 15 °C for seven days. If the colour of the cell culture medium changes from red to yellow indicating medium acidification, pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to ensure cell susceptibility to virus infection.

Titration every six months of frozen stocks of VHSV and IHNV is performed to verify the susceptibility of the cell cultures to infection.

4. *Microscopy*

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 × magnification. If obvious CPE is observed, virus identification procedures according to section IV have to be initiated immediately.

At the same time appropriate steps are taken to suspend the approved status of the production unit from where the virus positive sample originated as well as from any production units situated downstream.

Suspension of approved status has to be maintained until laboratory tests have proved the virus in question not to be VHSV or IHNV. A maximum of four weeks are allowed for identification of the virus, including the time needed for examination in reference laboratories.

5. *Subcultivation*

If no CPE has developed after incubation for seven days, subcultivation is performed to fresh cell cultures utilizing a cell area similar to that of the primary culture.

Aliquots of medium from all cultures/wells constituting the primary culture are pooled according to cell line following one freeze-thaw cycle of the cultures and 0,5 ml of the medium mixed with equal parts of antiserum to IPN as described in Section II.3. and the mixture incubated at 15 °C for 60 minutes. The mixture is then inoculated into homologous cell cultures at dilutions 1 : 1 and 1 : 100 as described in section III.2. The inoculation may be preceded by preincubation of the dilutions with divalent antiserum to IPN virus at appropriate dilution.

The inoculated cultures are then incubated for seven days at 15 °C with observation as in Section III.4.

IV. Virus identification

1. *Neutralization*

If evidence of CPE has been observed in a cell culture, medium is collected, cells removed by low speed centrifugation or membrane filtration (0.45 µm) and medium then diluted 1 : 100 and 1 : 10 000 in cell culture medium.

Aliquots of the dilutions are mixed and incubated for 60 minutes at 15 °C with equal parts of the following reagents separately :

— group specific antibody to VHSV (Egtved virus)	1 : 50 (*)
— group specific antibody to infectious hematopoietic necrosis virus (IHNV)	1 : 50 (*)
— specific divalent antiserum to infectious pancreatic necrosis virus (IPNV) (reference strains Sp and Ab)	1 : 50 (*)
— medium	1 : 1

(*) or as specified by the reference laboratory with regard to the possible cytotoxicity of the antisera.

The reagents used have to be of reference quality with regard to titre and specificity.

From each virus-serum mixture at least two cell cultures are inoculated with 50 UI each and then incubated at 15 °C. Development of CPE is checked as described in Section III.4.

If the test has not allowed safe identification of the virus within one week, one of the following steps has to be taken :

- transfer of the virus to a national fish virus reference laboratory for immediate identification ;
- application of immunofluorescence (IFAT) (Section IV.2.), enzyme-linked immunosorbent assay (ELISA) (Section IV.3.) or other virus identification techniques with reagents of reference quality.

2. Immunofluorescence (IFAT)

For each virus isolate to be identified, at least eight coverglasses or equivalent are seeded with EPC cells at a density leading to about 60 to 90 % confluency after 24 hours of cultivation. EPC cells are chosen for this purpose because of their strong adherence to glass surfaces.

When the cells have sedimented onto the glass surface (about one hour after seeding), or when the cultures have been incubated for up to 24 hours, the virus to be identified is inoculated. Four cultures are inoculated at a volume to volume ratio to 1:10, and four cultures at a ratio of 1:100.

Between 20 and 30 hours post inoculation, the cultures are rinsed twice in Eagle's MEM without serum, fixed in acetone and then stained by means of a two-layer IFAT. The first reagent layer consists of approved antiserum (as in Section IV.1.). The second reagent layer is an FITC-conjugated antiserum to the immunoglobulin used in the first layer. For each of the antisera tested at least one high-dose and one low-dose inoculated culture have to be stained. Proper negative and positive controls have to be included in the test.

Mount stained cultures using glycerol saline. Examine under incident ultraviolet (UV) light. Use 10 × or 12 × eyepieces and × 25 or × 40 objective lens with numerical apertures > 0,7 and > 1,3 respectively. Dilutions of primary antiserum and FITC conjugated anti-species immunoglobulin will depend on the microscope set-up chosen or available.

Some Egtved virus strains react strongly with antiserum to reference strain F 1 in IFAT although not reacting in neutralization tests.

3. Enzyme linked immunosorbent assay (ELISA)

Wells in microtiter plates (for instance Nunc-immunoplates, Maxisorp, Nunc, Denmark) are coated overnight with recommended dilutions of Protein-A purified immunoglobulin fractions of the antisera mentioned in Section IV.1.

After rinsing of wells with PBS-Tween-20 buffer, the virus to be identified is added to the wells in two or four-fold dilution steps and allowed to react with the coating antibody for 60 minutes at 37 °C. Following rinsing with PBS-Tween-20 buffer, biotinylated antibodies of a specificity corresponding to that of the coating antibodies are added and allowed to react for 60 minutes at 20 °C. Following another rinse as above, HRP conjugated streptavidin is added and allowed to react for one hour at 20 °C. After a last rinse, bound enzyme is visualized using appropriate ELISA substrates (OPD, TMB or others).

The above biotin-avidin based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

TABLE 1 A

Achievement of status

	No of clinical inspections per year	Examination of organs from number of fish	Examination of ovarian fluid from number of fish
<i>Continental zones:</i>			
(a) farms with broodstock	2	120 (1st series) 150 (2nd series)	30 (1st series) 0
(b) farms with broodstock only	2	0	150 (1st or 2nd series)
(c) farms without broodstock	2	150 (1st & 2nd series)	0
<i>Coastal zones:</i>			
(a) farms without broodstock	2	30 (1st & 2nd series)	0
(b) farms with broodstock	2	120 (1st series) 150 (2nd series)	30 (1st series) 0

Maximum number of fish per pool: 5

TABLE 1 B

Maintenance of status

	No of clinical inspections per year	Examination of organs from number of fish	Examination of ovarian fluid from number of fish
<i>Continental zones:</i>			
(a) farms with broodstock	2	15 (1st or 2nd series)	15 (1st or 2nd series)
(b) farms with broodstock only	2	0	30 (1st or 2nd series)
(c) farms without broodstock	2	30 (1st or 2nd series)	0
<i>Coastal zones:</i>			
(a) farms without broodstock	1	30 (1) (1st or 2nd series)	0
(b) farms with broodstock	2	0	30 (1st or 2nd series)

Maximum number of fish per pool: 10

(1) The samples have to be collected during the months two to six after transfer of fish from fresh to salt-water.

PART II:

DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION OF IHN AND VHS IN CASE OF SUSPECTED OUTBREAKS

Diagnosis of IHN and VHS can be achieved by one of the following techniques:

- A. Conventional virus isolation with subsequent serological virus identification.
- B. Virus isolation with simultaneous serological virus identification.
- C. Rapid diagnostic techniques (IFAT, ELISA).

The first diagnosis of IHN and VHS in farms in approved zones, must not be based on method C alone. Either method A or B must also be used.

The tissue material meant for virological examination in some cases may have to be accompanied by supplementary material for bacteriological, parasitological, histological or other examination to allow for a differential diagnosis. Such material is collected according to procedures outlined by OIE.

A. Conventional virus isolation with subsequent serological virus identification

A.I.1. Selection of samples

At least 10 fish showing typical signs of IHN or VHS must be selected for examination.

A.I.2. Preparation and shipment of samples from fish

Before shipment or transfer to the laboratory, pieces of the organs to be examined are removed from the fish with sterile scissors and forceps and transferred to plastic tubes containing transportation medium, i.e. cell culture medium with 10 % calf serum and antibiotics. The combination of 200 iu penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml can be recommended but other antibiotics of proven efficiency can be used as well. The organs to be examined are spleen, anterior kidney, and encephalon.

Organ pieces from 5 to 10 fish may be collected in one tube and represent one pooled sample. The tissue in each sample should weigh a minimum of 1g and such that the final dilution is about 1:10.

The tubes are placed in insulated containers (for instance thick-walled polystyrene boxes) together with sufficient ice or 'frost blocks' to ensure chilling of the samples to between 0 and 5 °C during transportation to the laboratory. Freezing must be avoided.

The virological examination must be started as soon as possible and not later than 48 hours after the collection of the samples. If the fish to be examined are less than 6 cm in length, the whole fish may be shipped to the laboratory in plastic bags chilled as mentioned above.

A.I.3. *Collection of supplementary diagnostic material*

According to agreement with the involved diagnostic laboratory, other fish tissues may be collected as well and prepared for supplementary examinations.

A.II.1. *Homogenization of organs*

In the laboratory the tissue in the tubes is completely homogenized (stomacher, blender or mortar and pestle) and subsequently the homogenate is suspended in the original transport medium. If a sample consisted of whole fish, i.e. fish less than 6 cm long, these are minced with sterile scissors after removal of the body behind the gut opening, homogenized as described above and suspended 1 : 10 in transport medium.

A.II.2. *Centrifugation of homogenate*

The homogenate is centrifuged in a refrigerated centrifuge at 2 to 5 °C at 2 000 to 4 000 × g for 15 minutes and the supernatant collected for examination.

If shipment of the sample has been made in transport medium (i.e. with exposure to antibiotics) the supernatant needs no further treatment with antibiotics.

If the sample was shipped as whole fish, the homogenate following centrifugation has to be exposed to the antibiotics in the transport medium used for resuspension for either four hours at room temperature or over night at 4 °C.

The antibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary. Immediately after the centrifugation a volume of the supernatant is mixed with equal parts of a suitably diluted divalent antiserum to IPN virus (reference strains Sp and Ab) and incubated with this for a minimum of one hour at 15 °C or a maximum of 18 hours at 4 °C. The titre of the antiserum must be at least 1 : 2 000 in a 50 % plaque neutralization test.

Treatment of all inocula with antiserum to IPN virus (a virus which in some parts of Europe occurs in up to 50 % of fish samples) aims at preventing CPE due to IPN virus from developing in inoculated cell cultures. This will reduce the duration of the virological examinations as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHS or IHN.

When samples come from production units which are considered free from IPN, treatment of inocula with antiserum to IPN virus may be omitted.

A.III.1. *Cell cultures and media*

BF-2 and either EPC or FHM cells are grown at 20 to 25 °C in Eagle's MEM (or modifications thereof) with a supplement of 10 % foetal bovine serum and antibiotics in standard concentrations.

When the cells are cultivated in closed vials, medium is buffered with bicarbonate. The medium used for cultivation of cells in open units has to be buffered with Tris-HCl (23 mM) and bicarbonate (6 mM) at a pH as close as possible to 7,6, a pH which is optimal for virus multiplication.

Cell cultures to be used for inoculation with tissue material should be young (four to 48 hours old) and actively growing (not confluent) at inoculation.

A.III.2. *Inoculation of cell cultures*

Antibiotic-treated organ suspension is inoculated into cell cultures in two dilutions, i.e. the primary dilution and in addition a 1 : 100 dilution thereof (in order to prevent homologous interference). At least two cell lines have to be inoculated (see A.III.1.).

The ratio between inoculum size and volume of cell culture medium should be about 1 : 10.

For each dilution and each cell line, a minimum of about 2 cm² cell area, corresponding to 1 well in a 24-well cell culture tray, has to be utilized. Use of cell culture trays is recommended, but other units of similar or bigger growth area are acceptable as well.

A.III.3. Incubation of cell cultures

Inoculated cell cultures are incubated at 15 °C for seven days. If the colour of the cell culture medium changes from red to yellow, indicating medium acidification pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to ensure cell susceptibility to virus infection.

Titration every six months of frozen stocks of VHS and IHN virus is performed to verify the susceptibility of the cell cultures to infection.

A.III.4. Microscopy

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 × magnification. If obvious CPE is observed, virus identification procedures according to Section AIV have to be initiated immediately.

At the same time appropriate steps are taken to suspend the approved status of the production unit from where the virus positive sample originated as well as from any production units situated downstream.

Suspension of approved status has to be maintained until laboratory tests have proved the virus in question not to be VHS or IHN. A maximum of four weeks are allowed for identification of the virus, including the time needed for examination in reference laboratories.

A.III.5. Subcultivation

If no CPE has developed after incubation for seven days, subcultivation is performed to fresh cell cultures utilizing a cell area similar to that of the primary culture.

Aliquots of medium from all cultures/wells constituting the primary culture are pooled according to cell line following one freeze-thaw cycle of the cultures and 0,5 ml of the medium mixed with equal parts of antiserum to IPN as described in Section A.II.2 and the mixture incubated at 15 °C for 60 minutes. The mixture is then inoculated into cell cultures at dilutions 1 : 1 and 1 : 100 as described in Section A.III.2. The inoculation may be preceded by preincubation of the dilutions with divalent antiserum to IPN virus at appropriate dilution.

The inoculated cultures are then incubated for seven days at 15 °C with observation as in section A.III.4.

A.IV.1. Neutralization

If evidence of CPE has been observed in a cell culture, medium is collected, cells removed by low speed centrifugation or membrane filtration (0,45 Uμ) and medium then diluted 1 : 100 and 1 : 10 000 in cell culture medium.

Aliquots of the dilutions are mixed and incubated for 60 minutes at 15 °C with equal parts of the following reagents separately :

— group specific antibody to VHS virus (Egtved virus)	1 : 50 (*)
— group specific antibody to infectious hematopoietic necrosis virus (IHN virus)	1 : 50 (*)
— specific divalent antiserum to infectious pancreatic necrosis virus (IPN virus) (reference strains Sp and Ab)	1 : 50 (*)
— medium	1 : 1

(*) or as specified by the reference laboratory with regard to the possible cytotoxicity of the antisera.

The reagents used have to be of reference quality with regard to titre and specificity.

From each virus-serum mixture at least two cell cultures are inoculated with 50 μl each and then incubated at 15 °C. Development of CPE is checked as described in section A.III.4.

If the test has not allowed safe identification of the virus within one week, one of the following steps has to be taken :

- Transfer of the virus to a national fish virus reference laboratory for immediate identification.
- Application of IFAT (section A.IV.2.), ELISA (section A.IV.3.) or other virus identification techniques with reagents of reference quality.

A.IV.2. Immunofluorescence (IFAT)

For each virus isolate to be identified at least eight coverglasses or equivalent are seeded with EPC cells at a density leading to about 60 to 90 % confluency after 24 hours of cultivation. EPC cells are chosen for this purpose because of their strong adherence to glass surfaces.

When the cells have sedimented into the glass surface (about one hour after seeding), or when the cultures have been incubated for up to 24 hours, the virus to be identified is inoculated. Four cultures are inoculated at a volume to volume ratio of 1 : 10, and four cultures at a ratio of 1 : 100.

Between 20 and 30 hours post inoculation the cultures are rinsed twice in Eagle's MEM without serum, fixed in acetone and then stained by means of a two-layer IFAT. The first reagent layer consists of approved antiserum (as in section IV.1.). The second reagent layer is an FITC-conjugated antiserum to the immunoglobulin used in the first layer. For each of the antisera tested at least one high-dose and one low-dose inoculated culture have to be stained. Proper negative and positive controls have to be included in the test.

Mount stained cultures using glycerol saline. Examine under incident UV light. Use 10 × or 12 × eyepieces and ×40 objective lens with numerical apertures >0.7 and >1.3 respectively. Dilutions of primary antiserum and FITC conjugated anti-species immunoglobulin will depend on the microscope set-up chosen or available.

Some Egtved virus strains react strongly with antiserum to reference strain F 1 in IFAT although not reacting in neutralization tests.

A.IV.3. Enzyme linked immunosorbent assay (ELISA)

Wells in microtiter plates (for instance Nunc- immunoplates, Maxisorp, Nunc, Denmark) are coated over night with recommended dilutions of Protein-A purified immunoglobulin fractions of the antisera mentioned in section A.IV.1.

After rinsing of wells with PBS-Tween-20 buffer, the virus to be identified is added to the wells in two- or four fold dilution steps and allowed to react with the coating antibody for 60 minutes at 37 °C. Following rinsing with PBS-Tween-20 buffer biotinylated antibodies of a specificity corresponding to that of the coating antibodies are added and allowed to react for 60 minutes at 20 °C. Following another rise as above HRP conjugated streptavidin is added and allowed to react for one hour at 20 °C. After a last rinse bound enzyme is visualized using appropriate ELISA substrates (OPD, TMB or others).

The above biotin-avidin based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

B. Virus isolation with simultaneous serological virus identification**B.I.1. Selection of samples**

As A.I.1-2.

B.I.2. Preparation and shipment of samples from fish

As A.I.3.

B.II.1. Homogenization of organs

As A.II.1.

B.II.2. Centrifugation of homogenate

As A.II.2.

B.II.3. Treatment of supernatant with diagnostic antisera

The antibiotic and anti-IPN treated organ suspension is diluted 1 : 10 and 1 : 10 000 in cell culture medium and aliquots mixed and incubated for 60 minutes at 15 °C with equal parts of the reagents listed in Section A.IV.1.

B.III.1. Cell cultures and media

As A.III.1.

B.III.2. *Inoculation of cell cultures*

From each virus serum mixture (prepared according to B.II.3.) at least two cell cultures per cell line are inoculated with 50 µl each.

B.III.3. *Incubation of cell cultures*

As A.III.3.

B.III.4. *Microscopy*

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 × magnification. If CPE is prevented by one of the antisera used, the virus can be considered to be identified accordingly.

If CPE is not prevented by any of the antisera, virus identification procedures according to A.IV. have to be performed.

B.III.5. *Subcultivation*

If no CPE has occurred after seven days, subcultivation has to be performed from cultures inoculated with supernatant plus medium (B.II.3.).

C. Rapid diagnostic techniques (IFAT, ELISA)

Supernatant prepared as described under A.II.2. is submitted to IFAT or ELISA according to A.IV.2. or A.IV.3., respectively. These rapid techniques have to be supplemented with a virological examination according to either A or B within 48 hours after sampling if:

(a) a negative result is obtained,

or

(b) a positive result is obtained with material representing the first case of IHN or VHS in approved zones.
