SN

中华人民共和国进出口商品检验行业标准

SN 0641—1997

出口肉及肉制品中丁烯磷残留量检验方法

Method for the determination of crotoxyphos residues in meats and meat products for export

1997-08-15发布　　1998-01-01实施

中华人民共和国国家进出口商品检验局　发布

前　言

本标准是根据GB/T 1.1—1993《标准化工作导则　第1单元：标准的起草与表述规则　第1部分：标准编写的基本规定》及SN/T 0001—1995《出口商品中农药、兽药残留量及生物毒素检验方法标准编写的基本规定》的要求进行编写的。其中测定方法是参考国内外有关文献，经研究、改进和验证后而制定的。本标准同时制定了抽样和制样方法。

测定低限是根据国际上对出口肉及肉制品中丁烯磷残留量的最高限量和测定方法的灵敏度而制定的。

本标准的附录A为提示的附录。

本标准由中华人民共和国国家进出口商品检验局提出并归口。

本标准由中华人民共和国上海进出口商品检验局负责起草。

本标准主要起草人：葛修丽。

本标准系首次发布的行业标准。

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1 范围

本标准规定了出口肉及肉制品中丁烯磷残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口猪肉中丁烯磷残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 2 500 件为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

批量，件	最低抽样数，件
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 抽样方法

按 2.2 规定的抽样件数，随机抽取，逐件开启。从每件中取一袋作为原始样品，其总量不少于 2 kg，放入清洁容器内，加封后，标明标记，及时送交实验室。

如每件中无小包装或有小包装但每袋重量超过 2 kg 者，则可用灭菌的锋利刀在抽出的包件中，每件割取不少于 100 g，混合后置于清洁容器内，作为混合原始样。混合原始样的重量不少于 2 kg。加封后，标明标记，及时送交实验室。

2.4 试样制备

从原始样品中分取出约 1 kg，经捣碎机充分捣碎，混匀，均分成两份，分别装入清洁的容器内，作为试样。加封并标明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样和制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

中华人民共和国国家进出口商品检验局 1997-08-15 批准　　1998-01-01 实施

3 测定方法

3.1 方法提要

试样中残留的丁烯磷用乙腈-水提取，提取液再用三氯甲烷液-液分配净化提取。三氯甲烷提取液经浓缩，残渣用正己烷浸出并经弗罗里硅土柱净化后，用配有火焰光度检测器的气相色谱仪测定，外标法定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水。

3.2.1 乙腈。

3.2.2 三氯甲烷：重蒸馏。

3.2.3 正己烷：重蒸馏。

3.2.4 丙酮：重蒸馏。

3.2.5 乙酸锌。

3.2.6 乙腈-水(1+1)。

3.2.7 丙酮-正己烷(5+95)。

3.2.8 丙酮-正己烷(20+80)。

3.2.9 无水硫酸钠：650℃灼烧 4 h，冷却后贮于密闭容器中备用。

3.2.10 助滤剂：Celite 545，使用前用乙腈-水(1+1)洗涤两次，每次浸泡半小时。

3.2.11 弗罗里硅土：80～100 目，650℃灼烧 4 h，冷却后贮于密闭容器中。使用前在 130℃下烘 4 h，冷却后加入 5%水脱活，加盖振摇，在密闭容器中存放 12 h 后并在 36 h 内使用。

3.2.12 硫酸钠溶液：20 g/L 水溶液。

3.2.13 丁烯磷标准品：纯度≥99%。

3.2.14 丁烯磷标准溶液：准确称取适量的丁烯磷标准品，用丙酮配制成浓度为 1.0 mg/mL 的标准储备液，再根据需要用正己烷稀释成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 气相色谱仪并配有火焰光度检测器，磷滤光片(526 nm)。

3.3.2 振荡器。

3.3.3 离心机。

3.3.4 真空旋转蒸发器。

3.3.5 超声波提取器。

3.3.6 混合器。

3.3.7 水浴锅。

3.3.8 布氏漏斗。

3.3.9 离心管：玻璃，具塞，50 mL。

3.3.10 层析柱：16 cm×1.6 cm(内径)，具砂芯。柱内先装 1.5 g 无水硫酸钠，再装 2.0 g 弗罗里硅土，顶部再装 2.5 g 无水硫酸钠。

3.3.11 微量注射器：10 μL。

3.4 测定步骤

3.4.1 提取

称取约 16 g 试样(精确至 0.1 g)于 150 mL 的锥形瓶中，加入 70 mL 乙腈-水(1+1)和 1.0 g 乙酸锌，加塞，先在振荡器上振荡 45 min。再放在超声波提取器中抽提 10 min。样液通过均匀铺盖约 1.5 g 助滤剂的布氏漏斗抽滤。用 3×10 mL 乙腈洗涤瓶中的残渣，每次放入超声波提取器中抽提 1 min，以后同上操作。合并滤液于 100 mL 容量瓶中，用乙腈-水(1+1)稀释至刻度。样液再经干滤纸快速过滤，弃去

前段 10 mL 滤液后收集约 30 mL 滤液。

准确量取 25 mL 滤液于离心管中，加入 10 mL 硫酸钠溶液及 5 mL 三氯甲烷。加塞在混合器上混合 2 min 后，于 3000 r/min 下离心 2 min。用滴管将下层三氯甲烷移入离心管中。于原溶液中再分别加入 3 mL、2 mL 三氯甲烷，以后同上操作。合并三氯甲烷层，在 50℃下，用旋转蒸发器减压浓缩近干后用空气流浓缩至干，加入 2 mL 正己烷以溶解残渣。

3.4.2 净化

在层析柱中，加入 20 mL 正己烷，待液面降至无水硫酸钠层时，将上述溶液加入柱中，待液面降至无水硫酸钠层时，用 5 mL 正己烷洗涤器皿，加入柱内，继续用 15 mL 丙酮-正己烷(5+95)淋洗，弃去以上流出液。最后用 30 mL 丙酮-正己烷(20+80)洗脱(流速约 1.5 mL/min)，收集洗脱液于离心管中，在 40℃下用旋转蒸发器减压蒸发至干。准确加入 1 mL 正己烷以溶解残渣，溶液供气相色谱测定。

3.4.3 测定

3.4.3.1 气相色谱条件

a) 色谱柱：石英毛细管柱，农残 1 号(兰州化学物理研究所)，25 m×0.53 mm(内径)，或相当者；

b) 载气：氮气，纯度≥99.99%，10 mL/min；

c) 尾吹气：氮气，纯度≥99.99%，20 mL/min；

d) 氢气：75 mL/min；

e) 空气：100 mL/min；

f) 色谱柱温度：程序升温，180℃保持 1 min，以 10℃/min 速度升至 220℃，恒温至峰出完；

g) 进样口温度：220℃；

h) 检测器温度：280℃；

i) 进样量：1～5 μL。

3.4.3.2 色谱测定

根据样液中丁烯磷含量情况，选定峰高与样液相近的标准工作溶液。标准工作液和样液中丁烯磷的响应值均应在仪器检测的线性范围内。标准工作液与样液应等体积参插进样测定。在上述色谱条件下，丁烯磷的保留时间约为 3.9 min。标准品的色谱图见附录 A 中图 A 1。

3.4.4 空白试验

除不称取试样外，均按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中丁烯磷的残留含量：

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \qquad \cdots\cdots(1)$$

式中：X——试样中丁烯磷残留含量，mg/kg；

h——样液中丁烯磷的色谱峰高，mm；

h_s——标准工作液中丁烯磷的色谱峰高，mm；

c——标准工作液中丁烯磷的浓度，μg/mL；

V——样液最终定容体积，mL；

m——最终样液所代表的试样量，g。

注：计算结果需将空白值扣除。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.02 mg/kg。

4.2 回收率

猪肉中丁烯磷的添加浓度及其回收率的实验数据：

0.02 mg/kg 时，回收率为 97.0%；

0.05 mg/kg 时，回收率为 100%；

2.00 mg/kg 时，回收率为 102%。

附 录 A
（提示的附录）
标准品色谱图



图 A 1　丁烯磷标准品的色谱图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993 "Directives for the work of standardization—Unit 1:Drafting and presentation of standards—Part 1:General rules for drafting standards" and SN/T 0001—1995 "General rules for drafting the standard methods for the determination of pesticide, veterinary drug residues and biotoxins in commodities for export". The method of determination of this standard was drafted by referring to relevant domestic and foreign literatures through research, modification and verification. In addition, methods of sampling and sample preparation are also specified in this standard.

The limit of determination in this standard is defined on the basis of the current international maximum limits for crotoxyphos residues in meats and meat products and the sensitivity of the method.

Annex A of this standard is an informative annex.

This standard was proposed by and is under the charge of the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by Shanghai Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafter of this standard is Ge Xiuli.

This standard is a professional standard promulgated for the first time.

Note: This English version, a translation from the Chinese text, is solely for guidance.

Professional Standard of the People's Republic of China
for Import and Export Commodity Inspection

Method for the determination of crotoxyphos residues in meats and meat products for export

SN 0641—1997

1 Scope

This standard specifies the methods of sampling, sample preparation and determination by gas chromatography of crotoxyphos residues in meats and meat products for export.

This standard is applicable to the determination of crotoxyphos residues in pork for export.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 2 500 packages.

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification, grade etc., should be the same.

2.2 Quantity of sample taken

Number of packages in each inspection lot	Minimum number of packages to be taken
1—25	1
26—100	5
101—250	10
251—500	15
501—1 000	17
1 001—2 500	20

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one. From each package, at least one bag shall be taken as a primary sample. The total weight of all the primary samples should not be less than 2 kg, which shall be placed in a clean container, sealed, labeled and sent to the laboratory in time.

In case the meat pieces are not contained in small bags inside each package, or if there are small bags inside but the content of bag exceeds 2 kg, cut out a part from the meat in each package of not less than 100 g with a disinfected sharp knife. Mix the parts of the meat as the mixed primary sample, which shall not be less than 2 kg. Place in a clean container, seal, label, and send to the laboratory in time.

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Aug. 15, 1997

Implemented from Jan. 1, 1998

2.4 Preparation of test sample

Reduce the combined primary sample to ca 1 kg. Blend in a blender and homogenize thoroughly. Mix and divide into two equal portions, each is placed in a clean container as the test sample, which is then sealed and labeled.

2.5 Storage of test sample

The test sample should be stored below −18℃.

Note: In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

The crotoxyphos residues in the test sample are extracted with acetonitrile-water. The extract is treated by chloroform liquid-liquid partitioning, and the chloroform extract is evaporated. The residue is dissolved in *n*-hexane, then the solution is cleaned up by passing through a florisil column. Determination is made by gas chromatograph with flame photometric detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, the reagents should be analytically pure, "water" is distilled water.

3.2.1 Acetonitrile.

3.2.2 Chloroform: Redistilled.

3.2.3 *n*-Hexane: Redistilled.

3.2.4 Acetone: Redistilled.

3.2.5 Zinc acetate.

3.2.6 Acetonitrile-water (1+1).

3.2.7 Acetone-*n*-hexane (5+95).

3.2.8 Acetone-*n*-hexane (20+80).

3.2.9 Anhydrous sodium sulfate: Ignite at 650℃ for 4 h, and keep in a tightly closed container.

3.2.10 Filter aid: Celite 545, wash twice with acetonitrile-water (1+1) before use. Each time let soak in the washing solution for 0.5 h.

3.2.11 Florisil: 80—100 mesh. Ignite at 650℃ for 4 h, and keep in a tightly closed container after cooling. Prior to use dry at 130℃ for 4 h, and after cooling deactivate by adding 5% of water. keep in a closed container for not less than 12 h and use within 36 h.

3.2.12 Sodium sulfate solution: 20 g/L aqueous solution.

3.2.13 Crotoxyphos standard: Purity≥99%.

3.2.14 Crotoxyphos standard solution: Accurately weigh an adequate amount of crotoxyphos standard, dissolve in acetone to prepare a solution of 1.0 mg/mL in concentration as the standard stock solution. According to the requirement, prepare a standard working solution of appropriate concentration by diluting the stock solution with *n*-hexane.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph, equipped with flame photometric detector and phosphorus filter (526 nm).

3.3.2 Shaker.

3.3.3 Centrifuge.

3.3.4 Rotary vacuum evaporator.

3.3.5 Ultrasonic extractor.

3.3.6 Mixer.

3.3.7 Water-bath.

3.3.8 Buchner funnel.

3.3.9 Centrifuge tube; Glass, with stopper, 50 mL.

3.3.10 Chromatographic column: 16 cm×1.6 cm(id), with sintered glass. Add in sequence 1.5 g of anhydrous sodium sulfate, 2 g of florisil and 2 g of anhydrous sodium sulfate.

3.3.11 Micro-syringe: 10 μL.

3.4 Procedure

3.4.1 Extraction

Weigh ca 16 g of the test sample(accurate to 0.1 g) into a 150 mL conical flask, add 70 mL of acetonitrile-water(1+1) and 1 g of zinc acetate, stopper and extract for 45 min on a shaker, then extract for 10 min in an ultrasonic extractor. Filter by suction through a Buchner funnel which is padded with 1.5 g of filter aid evenly. The residue in the flask is washed with 3×10 mL of acetonitrile. Each time extract for 1 min in ultrasonic extractor, then filter as above. Combine the filtrates into a 100 mL volumetric flask and make up to volume with acetonitrile-water(1+1). Filter the solution through a dry filter paper rapidly. Discard the first 10 mL of the filtrate, then collect ca 30 mL of the next.

Pipet exactly 25 mL of the filtrate into a centrifuge tube, add 10 mL of sodium sulfate solution and 5 mL of chloroform, stopper and mix violently in a mixer for 2 min. Centrifugalize at 3000 r/min for 2 min. Pipet the chloroform(low layer) into a centrifuge tube. Repeat the above procedure with 3 mL, 2 mL of chloroform. Combined the extracts and concentrate to near dryness on a rotary vacuum evaporator at 50℃, then evaporate to dryness under a stream of air. Dissolve the residue with 2 mL of *n*-hexane.

3.4.2 Cleanup

Rinse the chromatographic column with 20 mL of *n*-hexane. When the liquid level lowers to the upper surface of anhydrous sodium sulfate, pour the above solution into the column. Wash the container with 5 mL of *n*-hexane, and pour the washings into the column. When the solution drains to the surface of the sodium sulfate add 15 mL of acetone-*n*-hexane(5+95). Discard all the effluents. Elute with 30 mL of acetone-*n*-hexane(20+80) and collect the eluate into a centrifuge tube(controlling the flow rate at ca 1.5 mL/min). Evaporate to dryness on a rotary vacuum evaporator at 40℃. Add exactly 1 mL of *n*-hexane to dissolve the residue, the solution is ready for GC determination.

3.4.3 determination

3.4.3.1 GC operating condition

a) GC column: Fused-silica capillary column, NONGCAN No. 1 (Lanzhou Institute of Chemicophysics), 25 m×0.53 mm(id) or the equivalent;

b) Carrier gas: Nitrogen, purity≥99.99%, 10 mL/min;

c) Make up gas: Nitrogen, purity≥99.99%, 20 mL/min;

d) Hydrogen: 75 mL/min;

e) Air: 100 mL/min;

f) Column temperature: Temperature programmed: Keep at 180℃ for 1 min, then raise (10℃/min) to 220℃, keep at 220℃ until no more peak appears;

g) Injection port temperature: 220℃;

h) Detector temperacture:280℃;

i) Injection volume:1—5 μL.

3.4.3.2 GC determination

According to the approximate concentration of pesticide in the sample solution, select the standard working solution with similar peak height to that of the sample solution. The responses of crotoxyphos in the standard working solution and sample solution should be within the linear range of the instrumental detection. The standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. Under the above operating condition, the retention time of crotoxyphos is ca 3.9 min. For chromatogram of the standard see fig. A1 in annex A.

3.4.4 Blank test

The operation of blank test is the same as that described in the method of determination, but with the omission of sample addition.

3.5 Calculation and expression of result

Calculate the content of crotoxyphos residues in the test sample by GC data processor or according to formula(1):

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \qquad \cdots\cdots(1)$$

where

X—the residue content of crotoxyphos in test sample, mg/kg;

h—the peak height of crotoxyphos in the sample solution, mm;

h_s—the peak height of crotoxyphos in the standard working solution, mm;

c—the concentration of crotoxyphos in the standard working solution, μg/mL;

V—the final volume of the sample solution, mL;

m—the corresponding mass of test sample in the final sample solution, g.

Note: The blank value should be subtracted from the above result of calculation.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.02 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of crotoxyphos in pork and its corresponding recoveries are:

0.02 mg/kg, the recovery 97.0%;

0.05 mg/kg, the recovery 100%;

2.00 mg/kg, the recovery 102%.

Annex A

(informative)

Chromatogram of the standard



Fig. A1 GC chromatogram of crotoxyphos standard