Dissipation of the insecticides pirimiphos-methyl and dichlorvos in stored maize (Zea mays L.) grains

Disipación de los insecticidas pirimifós-metil y diclorvós en granos de maíz (Zea mays L.) almacenados

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ABSTRACT

The aim of this work was to analyze the dissipation dynamics of the insecticides pirimiphos-methyl and dichlorvos in maize (Zea mays L.) grains after post-harvest spray applications. The recommendations for their use for Argentina and the Maximum Residue Limits (MRLs) established by local and international pesticide regulations were considered. Supervised experimental trials were conducted in stored maize grains treated with 10 cc. t⁻¹ of pirimiphos-methyl and 20 cc. t⁻¹ of dichlorvos. Samples were monitored 2, 30, 60, 90 and 120 days after application. The pesticides were extracted from the maize grains using the QuEChERS technique and the residues were determined using high-resolution gas chromatography with mass detector. Residue dissipation percentages and daily dissipation rates differed between active ingredients. At 48 hours after application, pirimiphos-methyl residues were 5.1±0.42 μg. g⁻¹, i.e. below the MRLs established by the Argentine Service for Agrifood Health and Quality (10 μg. g⁻¹), the USA (8 μg. g⁻¹) and the Codex Alimentarius (7 μg. g⁻¹), and close to the MRLs established by the EU (5 μg. g⁻¹). Dichlorvos residues reached 2.97±0.27 μg. g⁻¹, and required at least a 90-day withholding period to be lower than the MRLs of the EU.

Keywords
Dissipation curves • Maximum Residue Limit • organophosphorus insecticides • pirimiphos-methyl • dichlorvos

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RESUMEN

El objetivo de este trabajo fue analizar la dinámica de disipación de los insecticidas pirimifós-metil y diclorvós aplicados durante el almacenamiento de granos de maíz (Zea mays L.). Se compararon los niveles de residuos recomendados para Argentina con el Límite Máximo de Residuos (LMR) establecido internacionalmente. Los ensayos fueron realizados en un ambiente controlado. Se aplicaron dosis de 10 cm$^3$.t$^{-1}$ de pirimifós-metil y 20 cm$^3$.t$^{-1}$ de diclorvós sobre granos de maíz almacenados. Las muestras se tomaron a los 2, 30, 60, 90 y 120 días desde la aplicación. La extracción de plaguicidas del tejido vegetal se realizó mediante la técnica QuEChERS y la determinación de residuos por cromatografía gaseosa de alta resolución con detector de masa. El porcentaje de disipación de residuos y la tasa diaria de disipación fue diferente para ambos principios activos. Los residuos de pirimifós-metil a las 48 horas de la aplicación fueron de 5,1±0,42 μg.g$^{-1}$, i.e., estuvieron por debajo de los LMR establecidos por SENASA (10 μg.g$^{-1}$), EUA (8 μg.g$^{-1}$), Codex Alimentarius (7 μg.g$^{-1}$) y cercanos a los LMR de UE (5 μg.g$^{-1}$). Para diclorvós los residuos a los 30 días alcanzaron valores de 2,97±0,27 μg.g$^{-1}$ y se requirieron al menos 90 días para alcanzar valores menores a los LMR de la UE.

Palabras clave

Curvas de disipación • Límite Máximo de Residuos • insecticidas organofosforados • pirimifós-metil • diclorvós

INTRODUCCIÓN

Grains from extensive crops such as maize are usually stored until commercialization. During storage, maize grains can be attacked by different pests, mainly insects, which can be controlled using insecticides. However, insecticides should be appropriately applied to avoid residues above the Maximum Residue Limits (MRLs) legally allowed by both national and international regulations (13). Proper pesticide application is mandatory, not only to prevent direct damage to grains, but also to avoid commercialization barriers, since the tolerance policy for live insects is nil (4). The insecticides usually applied to prevent pest attacks during storage belong to the chemical group of organophosphorus compounds (mercaptothion, dichlorvos, chlorpyrifos-methyl and pirimiphos-methyl) and pyrethroid compounds (permethrin, deltamethrin and lambda-cyhalothrin) (1, 6, 23, 27). Incorrect insecticide application can leave residues in the grains, generating health and commercialization problems. In Argentina, SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria) is the agency that regulates the safe use of pesticides and determines the MRLs.

Good agricultural practices in the management of pesticides in stored grains are mandatory. These practices include verifying that pesticide residues do not exceed the MRLs. A maximum residue level (MRL) is the highest level of a pesticide residue (expressed in μg.g$^{-1}$, mg.kg$^{-1}$ or ppm) that is legally tolerated in or on food or feed when pesticides are applied correctly; MRLs are intended to certify that the food and feed will be toxicologically accepted (7). On the other hand, the “withholding period” of a certain pesticide is the period that must elapse between the last application and crop consumption to ensure that residue levels are below the MRLs (5). The factors affecting degradation, persistence and amount of pesticide residues in grains include the storage environmental parameters (i.e. relative humidity and air temperature), type of grain substrate, type of pesticides (i.e. chemical group), the application dose, the technology used to apply the pesticides, and agricultural practices (7, 9). Since the MRLs vary according to the legislations of each country, at the moment of choosing the active ingredients it is necessary to consider not only the national quality standards but also the MRLs of the countries where the produced food and feed will be sent. There is evidence in the literature that after long periods of storage, the levels of pesticide residues in the grains decrease markedly, with reductions of up to 85% of the initial levels (16, 18), depending on the storage conditions (11, 22).
Objective

Analyze the dissipation dynamics of pirimiphos-methyl and dichlorvos applied in stored maize grains, using the guide for "Good Agricultural Practices" for the central region of Argentina. The final levels of insecticide residues obtained were compared with the MRLs established by national and international regulations.

Material and methods

The laboratory experiments were performed at the Estación Experimental Agraria del INTA (EEA INTA Manfredi), Córdoba province, Argentina. To simulate the storage conditions usually used for maize grains, 13 kg of grains were put in a 20-L plastic container that had perforated lids to allow gaseous exchange. Grain moisture content was 14%. The storage conditions were stable, with a temperature range between 20 and 25 °C, and relative humidity between 40% and 60%. The insecticides pirimiphos-methyl EC 50% and dichlorvos (DDVP) EC 100% were applied to the grains on the first day of the assay (day “0”) with a manual sprayer (Gerber 1.5 L). To apply the insecticides, the grains were placed on a 100-μm thick continuous sheet of polyethylene; thus, the insecticide was distributed homogeneously on this layer of grains. The dose applied was the maximum recommended on the commercial label of the product container: 10 cc.t⁻¹ in 500 cc of water for pirimiphos-methyl and 20 cc.t⁻¹ in 1000 cc of water for dichlorvos (5). The theoretical deposition of the active ingredient (g.t⁻¹) that remained in the grains immediately after application was calculated based on the concentration of active ingredient corresponding to each commercial formulation of the insecticides used for the assays (table 1). The experiments were performed using a complete random block design with three blocks. A control without pesticide application was included in each block.

After pesticide application, the grains were stored for 120 days and during this period, samples were taken using a sampler, following the guidelines established by the IRAM regulations (17) for residue pesticide sampling in cereals, oilseeds and their products. The grains were sampled on days 2, 30, 60 and 120 after application to quantify the residues present in the grains during the storage period and characterize the dissipation curve for each single active ingredient.

Grain samples were processed at the Laboratorio de Calidad de Granos at EEA INTA-Manfredi, Argentina. The milling was done with glass blenders. Insecticides were extracted from grains by using the QuEChERS technique (2, 19, 28, 29) adapted to dry matrices (20, 21).

Table 1. Active ingredients (a.i.), doses, theoretical deposition, application date, days and date of sampling to determine the insecticide residues in stored maize grains.

<table>
<thead>
<tr>
<th>Active ingredient (a.i.)</th>
<th>Theoretical dose (cc.t⁻¹)</th>
<th>Theoretical deposition of a.i.</th>
<th>Application date (day 0)</th>
<th>Days after application</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pirimiphos-methyl EC 50%</td>
<td>10</td>
<td>5</td>
<td>Sept. 1st</td>
<td>2</td>
<td>Sept 3rd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>Oct 1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>Nov 1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90</td>
<td>Dec 1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>120</td>
<td>Jan 5th</td>
</tr>
<tr>
<td>Dichlorvos EC 100%</td>
<td>20</td>
<td>20</td>
<td>Sept. 1st</td>
<td>2</td>
<td>Sept 3rd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>Oct 1st</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>Nov 1st</td>
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<td></td>
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<td>90</td>
<td>Dec 1st</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>120</td>
<td>Jan 5th</td>
</tr>
</tbody>
</table>
Each sample (5 g) was extracted in a centrifuge tube by adding water (10 mL) and acetonitrile (15 mL) and 150 µL of internal standard solution of ethoprophos (20 µg.mL⁻¹), 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride. The extract was then homogenized and centrifuged (RCF = 5000 g, 5 °C, 5 min). An aliquot (5 mL) of the organic phase supernatant was transferred to a clean-up tube containing 0.75 g of anhydrous magnesium sulfate, 0.2 g of PSA bulk sorbent and 0.2 g of C18, and then shaken and centrifuged (RCF = 5000 g, 5°C, 5 min). An aliquot of the supernatant (2 mL) was transferred and evaporated to dryness under a stream of nitrogen with the water bath set at 35°C. The sample was recovered with toluene (500 µL) and shaken; 300 µL was collected and placed in a vial. Then, 50 µL of TPP solution (2 µg.mL⁻¹) and 25 µL of toluene were added. Blank samples (without matrix) and fortified samples (with insecticide mixture) were prepared for quality control of the extraction technique.

Validations were also performed using calibration tests, matrix effect studies and recovery experiments to define the linear dynamic range and calculate recovery, expanded uncertainty, and limits of detection (LOD) and quantification (LOQ). Recovery data were used to assess the accuracy and precision of the method (Document SANCO 10684/2009; 26). Standard solutions of all the active pesticide compounds used in this study were prepared for validation assays by using mother solution concentrations of 1 mg.mL⁻¹ for DDVP, PMM and CPM, and 2 mg.mL⁻¹ for ethoprophos and TPP to derive working solutions. The fortification levels used for DDVP and PMM were 0.01, 0.05, 0.1, 1, 3 and 9 µg.g⁻¹, whereas those for CPM were 0.01, 0.05, 0.1, 1 and 3 µg.g⁻¹, using a matrix of organic maize flour (5 g) and three replicate tests per level.

Pesticide residues were determined by high-resolution gas chromatography with mass spectrometry (GC-MS) at the Laboratorio de Contaminantes Químicos del Instituto de Tecnología de Alimentos del CNIA of INTA Castelar. The gas chromatograph used was a Perkin Elmer model Clarus 600 coupled to a mass spectrometer with an electronic impact ionization source, quadrupole analyzer with a quadrupole pre-filter and a detector. Chromatographic separation was performed using a capillary column Varian model Factor Four VF-5ms of 30 m × 0.25 mm (id, 0.25 μm) of stationary phase (95%) dimethyl-(5%) slow bleeding polysiloxane biphenyl. The separation was carried out using Helium 5.0. The results were expressed in micrograms of pesticide per gram of plant material (µg.g⁻¹), which is equivalent to milligram per kilogram (mg.kg⁻¹) or parts per million (ppm).

Residue dissipation was analyzed using the repeated-measures ANOVA and the daily dissipation rates (DDR) were estimated. This parameter was expressed in micrograms of pesticide per gram of grain per day (µg.g⁻¹/day). The ANOVA model was adjusted as Mixed Linear Model (35), with treatment effects, days after application and their interaction being regarded as fixed effects, and including a random effect of experimental unit. The adjusted means of pesticide residue were compared with the LSD Fisher Test, with a significant level of 5%. The software InfoStat was used for statistical analysis (10). The time effect (days after applications) on the residue levels was analyzed for each active ingredient.

RESULTS AND DISCUSSION

Pirimiphos–methyl residues

The active ingredient pirimiphos-methyl belongs to the chemical group of organophosphorus compounds. According to the recommendations for its use, “no withholding period” is required (23). However, the long period of protection of this product was related to the persistence of its residues (20).

In the present study, the residue levels of pirimiphos–methyl in maize grain samples decreased significantly (p<0.05) after the day of application (table 2, page 409; figure 1, page 409).

The pirimiphos-methyl residue in maize grains found two days after application was similar to the initial theoretical deposition (5 µg.g⁻¹) according to the active ingredient percentage used in the dose, and was below the MRLs accepted by SENASA (10 µg.g⁻¹), the USA (8 µg.g⁻¹) and the Codex Alimentarius (7 µg.g⁻¹), and above the limit established by the EU (5 µg.g⁻¹). Likewise, 120 days after application, the residue was below the LOQ (0.012 µg.g⁻¹) (table 2, page 409).
Previous works reported lower DDR of pirimiphos-methyl in stored cereal grains (16, 18). For example, in stored cereals such as wheat, maize and rice, Singh & Chawla (1980) found 50% of the initial residue six months after application, whereas in stored maize grains, Yeboah et al. (1992) detected a decrease in the values of 13%, eight months after application. In stored wheat, other authors found residues between 35% and 54% of their initial concentration, at 127 to 270 days after application (3, 14, 15, 20, 37). Uygun et al. (2008) concluded that the storage period was not effective enough to reduce residues of pirimiphos-methyl below the established MRLs.

**Table 2.** Residue dissipation of pirimiphos-methyl in stored maize grains.

<table>
<thead>
<tr>
<th>Days after application</th>
<th>Residues (μg.g⁻¹) Mean ± SE*</th>
<th>Residue Dissipation (%)**</th>
<th>Daily Dissipation Rate (DDR) (μg.g⁻¹/day)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.10 ± 0.24 a</td>
<td>0.0</td>
<td>0.0560</td>
</tr>
<tr>
<td>30</td>
<td>3.54 ± 0.25 b</td>
<td>30.6</td>
<td>0.0670</td>
</tr>
<tr>
<td>60</td>
<td>1.52 ± 0.18 c</td>
<td>78.2</td>
<td>0.0027</td>
</tr>
<tr>
<td>90</td>
<td>0.72 ± 0.13 d</td>
<td>85.3</td>
<td>0.0024</td>
</tr>
<tr>
<td>120</td>
<td>&lt;LOQ</td>
<td>100.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*Different letters indicate statistically significant differences (p-value<0.05).
**Percentage of residue dissipation regarding the initial residue two days after application.
***Difference in the levels of residues between consecutive measurements divided by number of days of the study period.

LOQ: limit of quantification (0.012 μg.g⁻¹).

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**Figure 1.** Residue dissipation (%) (black squares) and level of residues (μg.g⁻¹) (white squares) of pirimiphos-methyl in stored maize grains 2, 30, 60, 90 and 120 days after application. Applied dose 10 cc.t⁻¹ active ingredient.

**Figura 1.** Disipación de residuos (%) (cuadrados negros) y nivel de residuos (μg.g⁻¹) (cuadrados blancos) de pirimifós-metil en granos de maíz almacenados a los 2, 30, 60, 90 y 120 días posteriores a la aplicación. Dosis aplicada: 10 cm³.t⁻¹ de principio activo.

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In studies of dissipation of pirimiphos-methyl in maize grains, Sgarbiero et al. (2003) reported the persistence of the insecticide during the first 30 days after application, with approximately 17% reduction, and an increase in the degradation between 30 and 60 days, with a reduction of about 56% of the initial residues; these values are lower than those found in the present study (70% reduction at 60 days) (figure 1, page 409).

Taking into account that the dissipation kinetics has three phases (a “stripping phase” mainly due to mechanical causes such as wind and rain; a “degradation phase” due to physical and chemical causes; and a “persistence phase” determined by the residues retained in the stored grain matrix [9]), it is possible to establish these phases for the DDR calculated for pirimiphos-methyl in maize grains (figure 1, page 409). The DDR values were higher from 2 to 30 days and from 30 to 60 days (0.056 μg.g⁻¹/day and 0.067 μg.g⁻¹/day respectively), coinciding with the “degradation phase”. These values then decreased over time, being 0.027 μg.g⁻¹/day between 60 and 90 days, and 0.024 μg.g⁻¹/day between 90 and 120 days after application (“persistence phase”).

**Dichlorvos residues**

Dichlorvos also belongs to the chemical group of organophosphorus compounds. It has a high steam tension that allows it to rapidly eliminate the insects by inhalation. This makes it appropriate for use in closed storage rooms. However, due to this property, it has little persistence, protecting stored grains for 15 days. According to the usage recommendations, the withholding period is established between 20 and 30 days after application, 20 days for the lower dose (10 cc.t⁻¹) and 30 days for the higher dose (20 cc.t⁻¹); the latter was the dose used in the present study (23).

The initial residue levels of dichlorvos found two days after application were lower than the expected theoretical deposition (20 g.t⁻¹), probably related to the higher volatility of this active ingredient (table 3 and figure 2, page 411).

In addition, two days after application, dichlorvos residues in maize grains exceeded the MRLs established by SENASA and the Codex Alimentarius (5 μg.g⁻¹). However, 30 days after application, the values decreased below these MRLs; thus, after the 30-day withholding period, dichlorvos residues would comply with the SENASA regulations (table 3). However, if these values are compared with the MRLs established by the EU, which is 0.01 μg.g⁻¹, considering the DDR, it would take more than 90 days to reach an adequate value of residues, which is important when the grains are destined for export markets. The residue values found at 120 days were below the LOQ of QuEChERS (0.006 μg.g⁻¹) (figure 2, page 411).

**Table 3. Dissipation dynamics of dichlorvos residues in stored maize grains.**

<table>
<thead>
<tr>
<th>Days after application</th>
<th>Residues (μg.g⁻¹) Mean ± SE*</th>
<th>Residue Dissipation (%) **</th>
<th>Daily Dissipation Rate (μg.g⁻¹/day) ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.27 ± 1.03 a</td>
<td>0.0</td>
<td>0.225</td>
</tr>
<tr>
<td>30</td>
<td>2.97 ± 0.16 b</td>
<td>68.0</td>
<td>0.086</td>
</tr>
<tr>
<td>60</td>
<td>0.38 ± 0.09 c</td>
<td>95.9</td>
<td>0.012</td>
</tr>
<tr>
<td>90</td>
<td>0.02 ± 0.01 c</td>
<td>99.8</td>
<td>0.001</td>
</tr>
<tr>
<td>120</td>
<td>&lt;LOQ</td>
<td>100.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*Different letters indicate statistically significant differences (p-value<0.05).

**Percentage of residue dissipation regarding the initial residue at two days after application.

***Difference in the levels of residues between consecutive measurements divided by the number of days of the study period.

LOQ: limit of quantification (0.006 μg.g⁻¹).

*Letras distintas indican diferencias estadísticamente significativas (valor-p <0,05).

**Porcentaje de desaparición de residuos respecto a los residuos iniciales a los 2 días después de la aplicación.

***Diferencia en los niveles de pirimifós-methyl entre mediciones consecutivas dividido el total de días transcurridos en el periodo.

LOQ: límite de cuantificación (0.006 μg.g⁻¹).
In previous studies, we reported levels of pesticide residues in samples of stored maize that were below the MRLs established by SENASA and the Codex Alimentarius (5 μg·g⁻¹) 60 and 90 days after application, confirming the influence of storage days on residue levels (33). In studies carried out in wheat, similar results were reported, with acceptable levels of residues under storage conditions at 25 °C and 12% humidity of grains (12), and residue levels lower than 5 ppm at 7 days and undetectable levels after 30 days of storage (8). However, considering these studies as reference should be done with caution due to the advance of analytical techniques to determine pesticide residues and the current greater requirement by safety standards.

The residue dissipation percentages were 68% at 30 days, 95.9% at 60 days, 99.8% at 90 days and 100% at 120 days after application (figure 2), which is in agreement with previous studies that showed a decrease in dichlorvos residues of 96% between 60 and 90 days after application (32). The DDR of the dichlorvos residues was greater between 2 and 30 days (0.225 μg·g⁻¹/day), which is considered the “degradation phase”, and decreased to 0.086 μg·g⁻¹/day between 30 and 60 days, 0.012 μg·g⁻¹/day between 60 and 90 days, and 0.01 μg·g⁻¹/day between 90 and 120 days, with the latter being considered the “persistence phase”. The half-life reported for dichlorvos in wheat is two weeks under storage conditions, at a temperature of 30 °C and 50% relative humidity (Desmarchelier cited by Holland et al. (1994). In this study, using the maximum DDR, the calculated average life was 20 days.

Although the levels of dichlorvos residues obtained in the present study were acceptable for the SENASA and Codex Alimentarius recommendations after the withholding period, in monitoring studies conducted in other countries, Ogah and Coker (2012) detected dichlorvos residues in 37% of the samples of commercial maize and found that 4% of them exceeded the established MRLs (2 mg·kg⁻¹ for cited study), indicating that these foods would not be safe. The fact that these foods contained amounts of residues exceeding the MRLs may be due to wrong technological practices in the application of these insecticides and might cause problems related to international trade considering the standard of each country.

To promote the competitiveness of Argentine production of grains for export, on May 7 2018 SENASA banned the import, commercialization and use of dichlorvos and formulations containing dichlorvos, for its use in grains, including the stages of production, postharvest, transport, handling, conditioning and storage, as well as in facilities for the storage of grains (SENASA Resolution No. 149/2018) (30). However, the study of dichlorvos
residues is important due to its wide use in developing countries and the evidence of persistence of its residues in the different matrices (25, 34).

The “withholding period” or “safety interval” in this experimental study for maize grains, considering the DDR calculated between 2 and 30 days (0.225 μg.g⁻¹/day), would be of 19 days after application to reach the MRL of 5 μg.g⁻¹, which coincides with the established withholding period (20 to 30 days) in the “Good Agricultural Practices of Application” and the MRLs for Argentina. However, if the grain consignments are destined for export, it would be necessary to consider the safety standards of other countries.

CONCLUSIONS

It is necessary to continue with the development and validation of techniques for the different matrices and active ingredients, performing local assays to determine the real dissipation curves for each pesticide.

The results provide a first approach to the study of organophosphorus insecticide degradation in maize grains stored under the usual conditions in central Argentina.

Insecticide application in stored grains should always follow the recommendations in the formulation label. The residue levels of the active ingredients studied decreased over time after application, but the percentages of residue dissipation and daily dissipation rate were different between pirimiphos-methyl and dichlorvos. The application of pirimiphos-methyl in stored maize grains, in the recommended dose, does not generate residues above the MRLs established by SENASA at 48 hours after application. The residues are below the limits established by the USA and the Codex Alimentarius, but exceeds the regulatory value established by the EU. The calculated theoretical withholding period in this case was four days. Dichlorvos applied to stored maize grains reached values lower than the MRLs established by SENASA after 30 days (withholding period). However, considering the MRLs established by the EU (0.01 μg.g⁻¹), dichlorvos residues would be above this maximum level allowed up to 90 days in the maize grains. For this reason, it is important to consider the legislation of the destination countries to avoid commercial problems with the products.

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