Global Monitoring Plan on Persistent Organic Pollutants

Standard Operating Procedure for the Sampling and Pre-treatment of National Samples within the UNEP/GEF Projects to Support the Global Monitoring Plan of POPs 2016-2019

March 2017

Basel Convention Coordinating Centre
Stockholm Convention Regional Centre
URUGUAY
Research Centre for Toxic Compounds in the Environment

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(Component 4
National Samples)

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For:

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1 SCOPE AND OBJECTIVES

This standardized operational procedure has been developed in the context of UNEP-implemented GEF projects to undertake capacity building and data generation in support of the Global Monitoring Plan of the Stockholm Convention (http://chm.pops.int/Convention/ConferenceofthePartiesCOP/Meetings/COP5/COP5Documents/tabid/1268/Default.aspx). It describes procedures for the sampling and analysis of so-called 'national samples'. A harmonized approach/guidance is needed to ensure that these samples are always taken correctly and treated in the same way. The present procedures describe sample selection, sampling, sample preparation, and dispatch to laboratories for POPs analysis.

Certain matrices/types of samples that will be analyzed by both the developing country laboratory and one of the reference laboratories are not covered in this guideline since they are part of a different component of the project. They have their own guides on sampling, sample handling or analysis as follows:

1. Polyurethane foams (PUFs) from passive air samplers
2. Human milk from primiparae mothers
3. Surface water samples for PFOS.

The objective of component 4 of the UNEP/GEF GMP2 projects is to (a) compare the analytical capability of the participating laboratories with that of the reference laboratories and (b) generate POPs data from samples relevant/chosen by the developing countries. This comes in addition to the interlaboratory assessment in which the laboratories can also test their analytical methods. However, in this 'mirror' study countries/laboratories can select their own samples and share them with the reference laboratory. This enables the laboratories to carry out a comparison on samples in which they have built up routine, which is not always the case for test materials in the interlab studies.

The results of this mirror exercise will help to identify and understand possible differences between the analytical methods for POPs used in the participating countries and the reference laboratories.

2 APPROACH

Countries participating in the UNEP GMP2 projects 2016-2019 are requested to select a number of samples that will be analyzed in the international expert laboratory and where possible in the developing country laboratory as well. In the latter case, this is a mirror analysis where two laboratories analyse the same sample and compare the results. For mirror analysis, it is not necessary that the same analytical method is used (*e.g.,* extraction, cleanup methods, or instrumentation can be different).

As this project may deliver a number of interesting data for persistent organic pollutants

(POPs), it is tempting to use these data for interpretation of geographical and temporal trends. Therefore, the objectives for selecting the samples – type, geography, *etc*. – must be clear before starting any sampling. Objectives may include the following:

1. Study/define human exposure of the general population through foodstuffs or living conditions.

2. Study environmental situation through analysis of abiotic or biotic matrices (sediments, indoor air, certain vegetation – grass, pine needs – wild fish or crabs).

It shall be noted that it is not the objective of the GMP projects to (i) identify hot-spots (soil, sediment) or (ii) undertake source inventories (*e.g.,* measurements of emissions/releases) or products such as television sets, pesticide mixtures.

Further, it shall be noted that samples must be easily accessible and not restricted; for example samples from wildlife may not only be difficult to interpret but also be prohibited for sampling.

However, as samples provided by various countries may be rather heterogeneous, most data will only give information on POPs in the local situation and cannot be used for any geographical trend analysis. To enable at least some observations on geographical differences, we recommend that all participants at least include one local fish sample in this study; see Section 4 for other types of sample selection.

It is essential that the sample received at the laboratory conserves its integrity. When the sample serves as a mirror sample, *i.e.,* is being analyzed by the reference laboratory and a national laboratory, it should be identical for both. The participating country is responsible for the selection of the samples and sampling, as well as ensuring the integrity of each sample, no matter if a mirror sample or single sample to generate new data on the presence of POPs in one of the participating countries. This refers to the sample matrix as well as to the POPs therein.

3 SAMPLE SELECTION, SAMPLING AND PRE-TREATMENT

3.1 General

Before the start of any POPs analysis, an adequate study design has to be established to ensure that the sampling and subsequent analysis will meet the objectives of the project. Therefore, close cooperation between the reference laboratory and the participating country needs to be established to understand the objectives of the project and adequately accommodate all needs. All activities should be conducted by trained professionals, according to a well-designed plan and using internationally or nationally approved methods, carrying out the same method each time over the time span of the project. It should be understood that mistakes in sampling or analysis – and reporting or storage of data or any deviation from standard operational procedures can result in meaningless data or even project-damaging data.

Quality control and quality assurance are important factors both in sampling and analysis. All

steps need to be documented.

A pre-determined number and type of samples should be selected, sampled, and sent to the reference laboratory and analyzed either by both the participating country laboratory and the reference laboratory or in cases where no national laboratory exists/is capable for such analysis, only the reference laboratory will provide results.

Any sampling plan shall be discussed with the analytical laboratory/laboratories. Contacts will be made by the reference laboratories between 1 March 2017 and 1 July 2017.

Sample size should be large enough to allow a duplicate or triplicate analysis in each laboratory. Samples should not include imported matrices from another country.

3.2 Materials for sampling, shipment or analysis

3.2.1 Sampling

With respect to sampling indispensable requirements include:

a) Equipment and materials: To have adequate sampling instruments according to the type of matrix and POP (dredger, spoons/shuffles, amber glass jars, plastic bags, aluminum foil, steel bowls for mixing in the field, knives, *etc.*);

b) Personal protection: Those in charge of the sampling must wear adequate protection outfits depending on the type of samples they will work with (gloves, rubber boots, goggles, *etc.*);

c) Sample blanks: These allow for the assessment of potential contamination;

d) Preservation: Samples and sample blanks have to be preserved according to matrix and type of POPs requirements;

e) Transportation: Adequate transportation that minimizes the possibility to contaminate the sample, ensuring its integrity and conservation until it reaches the laboratory in charge of the analysis;

f) Availability of "*in situ*" monitoring equipment: To measure or record relevant environmental parameters according to each environment. The environmental conditions should be registered;

g) Geo-referencing or photographic registers: Availability of GPS to locate sampling sites with precision and ensure future location of the site;

h) Standardized protocol: Well-established sampling procedures have to be applied;

i) Labelling: Unambiguous labels are needed.

If the country prefers to have local comparison, then, they must describe the discriminator and why an (opposite) pair of samples was taken. A good characteristic of the sample and the representativeness are important.

3.2.2 Recommended Sample Matrices

It shall be noted that samples have to be specific for the analytes; certain conditions apply such as amount of material necessary for analysis, packaging/shipment, and suitability.

Due to the suitability/relevance of fish for all POPs, it is recommended that each country selects at least one fish sample for analysis across all POPs.

The following recommendations for matrix selection are given:

a) OCPs and indicator PCB:
   - Abiotic matrices: Sediment
   - Biota: Fish, shellfish, (chicken) eggs, beef, …

b) Dioxin-like POPs:
   - Abiotic matrices: Sediment, indoor air (if passive air samplers are available), respirator dust
   - Biota: Fish, shellfish, (chicken) eggs, butter, beef; pine needles
     Lamb, pork, turkey or chicken meat are not recommended since concentrations are expected to be low due to low age of the animals, limited exposure or low fat content of the matrix

c) Brominated Flame Retardants
   - Abiotic matrices: Sediment, indoor air (if passive air samplers are available), respirator dust
   - Biota: Fish, shellfish, (chicken) eggs, beef

d) PFOS
   - Abiotic matrices: Sediment
   - Biota: Fish, (chicken) eggs.
     Shellfish and other meat is not recommended since concentrations are expected to be below detection limits.

3.2.3 Storage and Transport

Before selecting samples, the possibilities for shipping these materials to the analytical laboratory – no matter if domestic or abroad – have to be carefully evaluated. Especially biota may have a short period of expiration and may need cooling or freezing during transport or storage. Therefore, the timely accessibility and availability of fridges, freezers, icepacks, dry ice, of lyophilsators, thermos bottles, *etc*. has to be decided and agreed in advance.

3.2.3.1 General Requirements

For any shipment using a carrier, polystyrene boxes shall be used. These have to be properly declared as to content of the materials and required declaration.

Most sample matrices can be shipped in (pre-cleaned) amber glass jars. Please close them carefully and place a label containing a unique identifier on the outside.

When shipping biotic samples, make sure that samples are kept cold during transport; place label on box so that cooling is not interrupted (at airports).

For the UNEP GMP2 project, agreements between the expert laboratories and DHL exist and are recommended to be used.

Provisions for adequate storage. These conditions are dependent on the analyte and the matrix, but in general, the following conditions and times are proposed:

- Biota and other solid samples: Refrigerator at – 20 °C;
- Adequate storage also includes:
- Registry of the performance of refrigerators and freezers, e.g., registration and control of temperature;
- Availability of automatic power-supply equipment in case of power cuts;

3.2.3.2 Shipment of Samples Deep-Frozen

This method of keeping a sample frozen is recommended for many biota, especially after homogenisation.

3.2.3.3 Shipment of Samples on Dry Ice

Cooling options to use dry ice is recommended for biotic materials, especially fresh foodstuff having a short expiration date. However, restrictions apply such as:

- Importer/exporter has to be accredited by the shipment company
- Certain countries have restrictions (for information, a list including the 43 countries taking part in the UNEP GMP2 projects with status September 2016 is contained in section ANNEX 3. Restrictions for Shipment Using Dry Ice).

3.2.3.4 Shipment of Samples in a Thermos

Thermos are available at different sizes; are more efficient than icepacks.

Take care that they do not break during (air) transport.

3.2.3.5 Lyophilsation before Shipment

If a lyophilisator is available and cross-contamination can be excluded, freeze-drying the sample is a viable option. Lyophilized samples shall be kept in amber glass jars; there is no need for further cooling.

3.2.4 Pretreatment

Mirror samples need to be homogenized before splitting the sample and sending one portion of this sample to the reference laboratory and the other to the domestic laboratory. During pre-treatment, either filleting, homogenization, or freeze-drying, utmost care should be taken to avoid contamination of the sample. Contamination can easily occur through dust, plastics, paper, dirty knives, etc.

The reference laboratory will not apply any further treatment to the sample but analyze the sample as received. That means, if a sample arrives deep frozen, it will be thawed, homogenized and an aliquot will be taken into analysis. If the sample arrives as a dried or freeze-dried material, it will be homogenized and taken into analysis as such. The following pre-treatment procedures should be applied.

3.3 Abiotic samples

3.3.1 Sediment or Soil

Sediment or soil should contain sufficient organic carbon to allow a proper analysis of POPs. That means that sandy matrixes should not be used. The sample should be free of roots and other coarse materials. The sample should preferable be sieved using a 2 mm sieve. The sample should be homogenized and, after splitting into two parts, one part should be sent as such and one part used as such in the own laboratory. After an initial homogenization, the sediment and soil should be air dried (not in an oven). Subsequently, the sample should be homogenized again and then split into two parts, one for the own laboratory and one to be sent to the reference laboratory. The samples can be sent without cooling.

Sediment or soil can for example be homogenized in a steel/aluminum bowl and then split into two glass jars/plastic bottles, closed and shipped. No need to sieve all samples before distribution.

Soil samples preferable are from arable soil (no grassland, pasture), taken at a depth up to 30 cm.

Recommended amounts:

For OCPs and indicator PCB:	Samples should contain at least 1 gram of organic carbon, corresponding with a minimum of 50 g sediment.

For dl-POPs:	**at least 10 g of organic carbon corresponding to at least 50 g of sediment.**
For PFOS:	**at least 25 g of sediment Soil is not recommended**

3.3.2. Vegetables, Maize or Comparable Samples

This matrix may be interesting for organochlorine pesticides, but not for dioxin-like POPs, polybrominated flame retardants or PFOS.

Vegetable samples should preferably be taken from crop at the time of harvest. Only edible parts should be used. Vegetables can also be bought in a shop or super market but should be local and not imported from another country. Vegetables should be sent deep frozen, but if not possible, they can be freeze-dried. As regards quantities, each sub-sample should be ca. 500 g, as lipid contents may be low and lower sample amounts may cause relatively high detection limits and, consequently, many non-detects, which is not meaningful for this exercise. First homogenize in a mixer, then split into two and send deep frozen, or first homogenize, freeze-dry, homogenize again, split into two parts and send to the laboratories.

3.4 Biotic samples

Food items: When selecting foodstuff, consideration can be given to sample commonly consumed foodstuffs, *e.g.*, foodstuffs bought in the supermarket of the most consumed brands. Some matrices such as butter, do not to be homogenized before shipment and analysis; *e.g.* a pack of butter can be split into two and sent.

3.4.1. Eggs

Chicken eggs can be from large farms or small housing. Indicate raising procedures (free-ranging, *etc.*).

Chicken eggs can be placed in a clean amber glass jar and deep-frozen prior to shipment.

A homogenized sample is prepared by placing 8 or more eggs into a mixer, mix well and then divide into two parts. Each sub-sample should consist of 4 eggs, *e.g.*, *ca.* 100 g to 150 g wet weight.

For PFOS analysis, eggs shall be transported and stored in pre-cleaned HDPE bottles.

3.4.2. Fish

The fish sample may serve different purposes to be defined before sampling. Therefore, information such as important for consumption, geography, size/species, trophic level, fresh-water or salt-water fish, farmed vs. wild fish should be collected. There are different options:

Pooled fish muscle homogenate based on ca. five fishes of the same species and the same

location if caught in the wild. Fish may be bought at a local market or super market. In case of catching wild fish, the sampling location should be recorded. Organs, skin, bones, tail and head should not be used.

The fish should be filleted and the muscle tissue should be thoroughly homogenized. Before homogenization, any blood on the fillet should be washed away with distilled water. The homogenate should be split into two parts, of which one is used for the own laboratory and one is sent deep frozen to the reference laboratory. If the fish sample cannot be sent deep frozen, the sample can be freeze-dried. In that case, after filleting the fish sample should be homogenized, freeze-dried, homogenized again and then split into two parts, one for the own laboratory and one to be sent to the reference laboratory. The moisture and lipid content should always be determined in the homogenate and the method of the moisture and lipid determination should be reported.

Smoked fish is not recommended.

As regards the volume, the following minimum amounts of sample *per* sample and *per* laboratory are recommended:

For OCPs and indicator PCB:	At least 1 gram of lipids As many fishes have a fat content of ca 1%, this means the initial sample should be ca. 100 g. In case fat contents are higher, less material can be used.
For dl-POPs:	at least 10 g of lipids; corresponding to ca. 1000 of lean fish or ca. 100 g of fatty fish
For PFOS:	at least 50g of fish, wet weight

3.4.1 Butter

It is recommended to buy a pack of ca. 250 g of local (not imported) butter wrapped in aluminum foil and ship it to the laboratory without any further treatment. After purchase, samples should be deep-frozen until shipment.

3.5 Other samples

For any other types of samples, such as different types of meat, the principles should be the same as described above. That means, there should be a reasonable quantity that allows the analysis in duplicate or triplicate in each sub-sample. Homogeneity is a key issue, and should be paid much attention to according to the examples described above. Sample types such as yam, most corn types, rice and rice straw should be avoided as those normally do not contain measurable amounts of POPs. Also, ash, dust, wipe samples or products such as carpets should not be used. Some samples, such as butter (see above), do not need any pre-treatment as they are already homogeneous. In case you have an interest in indoor air, and you have two PUFs available, you can sample indoor air for four weeks and send one PUF while analyzing

the second PUF yourself.

4 DISPATCH

All materials should be sent in glass screw capped jars. The jars should be very carefully packed to avoid breakage underway. They should be pre-cleaned by rinsing warm water and detergent, ethanol and with pentane or hexane. The jars should be clearly labeled. The reference laboratory where to send the samples will be made known to you. Contact details of the reference laboratories are given in Par. 6. As all reference laboratories are in Europe, attention should be paid to the European import guidelines. These guidelines may change from time to time, so it is necessary to check these before dispatch. Please see: http://ec.europa.eu/food/safety/international_affairs/trade/index_en.htm.

You are requested to email the reference laboratory as soon as you have sent the samples with an indication of the time of arrival of the samples in the reference laboratory. An example of an accompanying letter to the sample to inform customs is given in Par. 7. You are requested to send the samples by a courier service and not by regular mail. The form shown in Annex 2 should be completed with relevant details of each mirror sample, such as data on the collection, moisture content, etc. Annex 3 shows restrictions per country for using dry ice during shipment.

5 START OF ANALYSIS

Until analysis you should ensure a proper storage of your sample. Freeze-dried samples can be stored under ambient conditions in a screw capped glass jar. When starting the analysis the material should first be homogenized. Fresh materials, apart from sediments, should be stored at –20 ˚C. Those materials should be thawed before analysis and homogenized. An aliquot of the freeze-dried or fresh material should be taken into analysis. This should be done immediately after homogenization. Further information on the analysis of the various POPs including sample intake quantities can be found in the specific SOPs.

6 REFERENCE LABORATORIES

The contact details of the three reference laboratories are:

Örebro University, School of Science and Technology
MTM Research Centre
Prof.Dr. H. Fiedler
SE-701 82 Örebro
Sweden
Tel.: +46 19303153
E-mail: Heidelore.fiedler@oru.se
Skype: heidifiedler

Vrije Universiteit Amsterdam
Department Environment & Health
Prof.Dr. Jacob de Boer
De Boeleaan 1108
1081HZ Amsterdam
The Netherlands
Tel.: +31 20 5989530
Fax: +31 20 5989553
Email: jacob.de.boer@vu.nl
Skype: jacobdeboer2

Spanish National Research Council (CSIC)
Laboratory of Dioxins
Institute of Environmental Assessment and Water Research (IDAEA)
Dr. Esteban Abad
Jordi Girona 18-26
E-08034 Barcelona
Spain
Tel.: + 34 93 4006185
Fax: + 34 93 2045904
Email: esteban.abad@idaea.csic.es
Skype: eaheco

7 CUSTOMS LETTERS

Declaration to be agreed before shipment.

UNITED NATIONS ENVIRONMENT PROGRAMME
Programme des Nations Unies pour l'environnement Programa de las Naciones Unidas para el Medio Ambiente
Программа Организации Объединенных Наций по окружающей среде برنامج الأمم المتحدة للبيئة
联合国环境规划署
UNEP

Date: 15 March 2017

Subject: Statement of non-commercial nature of shipment of national samples from developing countries to expert laboratories for capacity building and training purposes

To Whom It May Concern

Please note that UN Environment is implementing capacity building projects in three UN regions and has committed 42 developing countries to send national samples (maximum of 1 kg per sample) to one of three expert laboratories located in Spain, Sweden or the Netherlands to be analysed for persistent organic pollutants. The list of developing countries and related expert laboratories is provided in Annex 1.

The samples in the developing country of origin have been collected following agreed and UN-approved protocols and the shipment has been notified between the developing country and the recipient expert laboratory. The results from the analyses will be used for the evaluation of the effectiveness of measures taken by countries under the Stockholm Convention on Persistent Organic Pollutants. Through the above-mentioned regional projects, capacity for environmental monitoring of persistent organic pollutants (POPs) are built as part of the country's obligation under the convention.

The national samples contained in this shipment have no commercial value, are not for human consumption, and are not toxic.

To avoid further delays, my contact information is shown below

Jacqueline Alvarez
Science and Risk Unit Leader

United Nations Environment Programme
Chemicals and Waste Branch, Economy Division
11-13 chemin des Anémones
CH-1219 Châtelaine (GE)
Switzerland
Phone: +41 22 917 8350
Email: jacqueline.alvarez@unep.org

Annex 1

Africa region: Countries of origin are shown below. Recipient laboratories are:

- VU University Amsterdam, Environment and Health (E&H), De Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands
- Örebro University, School of Science and Technology, Man-Technology-Environment Research Center (MTM), SE-701 82 Örebro, Sweden

- DR Congo
- Egypt
- Ethiopia
- Ghana
- Kenya
- Mali
- Morocco
- Mauritius
- Nigeria
- Senegal
- Tanzania
- Togo
- Tunisia
- Uganda
- Zambia

Asia region: Countries of origin are shown below. Recipient laboratories are:

- VU University Amsterdam, Environment and Health (E&H), De Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands
- Örebro University, School of Science and Technology, Man-Technology-Environment Research Center (MTM), SE-701 82 Örebro, Sweden

- Cambodia
- Indonesia
- Lao PDR
- Mongolia
- Philippines
- Thailand
- Vietnam

Pacific Islands region: Countries of origin are shown below. Recipient laboratories are:

- VU University Amsterdam, Environment and Health (E&H), De Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands
- Örebro University, School of Science and Technology, Man-Technology-Environment Research Center (MTM), SE-701 82 Örebro, Sweden

- Fiji
- Kiribati
- Marshall Islands
- Niue
- Palau
- Samoa
- Solomon Islands
- Tuvalu
- Vanuatu

GRULAC region: Countries of origin are shown below. Recipient laboratories are:

- Spanish National Research Council (CSIC), Laboratory of Dioxins, Institute of Environmental Assessment and Water Research (IDAEA), Jordi Girona 18-26, E-08034 Barcelona, Spain
- Örebro University, School of Science and Technology, Man-Technology-Environment Research Center (MTM), SE-701 82 Örebro, Sweden

- Antigua and Barbuda
- Argentina
- Barbados
- Brazil
- Chile
- Colombia
- Ecuador
- Jamaica
- Mexico
- Peru
- Uruguay

Africa region: Countries of origin are shown below. Recipient laboratories are:

- Vrije Universiteit Amsterdam, Environment and Health (E&H), De Boelelaan 1108, NL-1081 HZ Amsterdam, The Netherlands
- Örebro University, School of Science and Technology, Man-Technology-Environment Research Center (MTM), SE-701 82 Örebro, Sweden

- DR Congo
- Egypt
- Ethiopia
- Ghana
- Kenya
- Mali
- Morocco
- Mauritius
- Nigeria
- Senegal
- Tanzania
- Togo
- Tunisia
- Uganda
- Zambia

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- Indonesia
- Lao PDR
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- Vietnam

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- Antigua and Barbuda
- Argentina
- Barbados
- Brazil
- Chile
- Colombia
- Ecuador
- Jamaica
- Mexico
- Peru
- Uruguay

ANNEX 2. INFORMATION ON COLLECTED SAMPLES (TO BE COMPLETED FOR EACH SAMPLE SENT)

Name of laboratory:

Address of laboratory:

Country:

Email address:

Tel. no.:

Name of responsible scientist:

Sampling code:

Type of sample (e.g., fish, vegetable, …etc.):

Sampling date:

Sampling location (field data GIS or shop/town):

Total weight sampled:

Weight of sub-sample sent to reference laboratory:

No. of samples (e.g., eggs, fish) in pooled sample (if relevant):

Dried/ deep frozen/ freeze dried/ no pre-treatment:

Date of dispatch to reference laboratory:

Specific observations:

ANNEX 3. RESTRICTIONS FOR SHIPMENT USING DRY ICE

With status September 2016 and according to information from DHL, the shipment on dry ice is either allowed or not allowed to participating countries as shown in the tables below.

Africa

Country	Dry ice allowed (Yes/No)
Congo	No
Egypt	No
Ethiopia	No
Ghana	Yes
Kenya	No
Mali	No
Morocco	Yes
Nigeria	Yes
Senegal	Yes
Tunisia	No
Tanzania	No
Zambia	No

Asia

Country	Dry ice allowed (Yes/No)
Indonesia	Yes
Cambodia	No
Mongolia	No
Philippines	Yes
Vietnam	Yes

Pacific Islands

Country	Dry ice allowed (Yes/No)
Fiji	No
Kiribati	No
Marshall Islands	No
Niue	No
Palau	No
Samoa	No
Solomon Islands	No
Tuvalu,	No
Vanuatu	No

GRULAC

Country	Dry ice allowed (Yes/No)
Argentina	Yes (Accepted with conditions: Dry ice shipments are limited to 55 lb (25 kg) per package)
Antigua	No
Brasilia	No
Barbados	No
Chile	Yes (Accepted with conditions: Dry ice shipments are limited to 55 lb (25 kg) per package)
Colombia	Yes (Accepted with conditions: Dry ice shipments are limited to 55 lb (25 kg) per package.)
Ecuador	Yes (Accepted with conditions: Dry ice shipments are limited to 55 lb (25 kg) per package.)
Jamaica	Yes
Mexico	Yes
Peru	Yes (Accepted with conditions: Dry ice shipments are limited to 55 lb (25 kg) per package.)
Uruguay	No