Environment Canada Environnement Canada

NSN TECHNICAL GUIDANCE SERIES

New Substances Notification

OECD Test Guideline 120 for
WATER EXTRACTABILITY OF POLYMERS

Canada

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The New Substances Notification (NSN) Technical Guidance Series has been developed to help address specific information requirements of the *New Substances Notification Regulations (Chemicals and Polymers)* (the Regulations) of the *Canadian Environmental Protection Act, 1999* (the Act).

This document addresses technical issues related to the water extractability test for polymers prescribed under schedules 10 and 11 of the Regulations. It introduces the Organisation for Economic Co-operation and Development's (OECD) Guideline 120 as a replacement to OECD Test Guideline 105 in those schedules and also provides guidance on interpretation and procedures.

1. Background

A water extractability test was not an information requirement within the original *New Substances Notification Regulations* (NSNR) published in July 1994. Water solubility (OECD Test Guideline 105) was required, but often resulted in technical challenges and questionable results for polymers. Since water extractability is more appropriate to polymers, the water solubility requirement was replaced by water extractability when the NSNR were revised in 2005. In addition, the ecotoxicity, biodegradation and hydrolysis requirements in schedules 10 and 11 now depend on the polymer's water extractability.

2. Water Availability versus Water Extractability

While discrete chemicals can form true thermodynamic solutions, polymers, by virtue of their molecular weight distribution, often result in the formation of heterogeneous mixtures. The smaller molecular components of a polymer may dissolve completely, whereas the larger components may form emulsions, dispersions or gels, or remain in suspension. Although emulsions, dispersions and gels are not truly dissolved, they are still "available" for potential exposure to humans and aquatic organisms and other wildlife. For assessment purposes the term "water availability" describes the behaviour of polymers in an aqueous environment more appropriately.

The water extractability test described by OECD Test Guideline 120 provides information used to assess the water availability of polymeric substances.

3. OECD Test Guidelines

OECD Test Guideline 105

OECD Test Guideline 105 is a method that was developed to test the water solubility of pure chemicals. The preliminary test of this method contains a stepwise procedure in which increasing volumes of water are added to a sample in an attempt to find a volume in which complete dissolution of the chemical occurs. This testing assumes formation of a saturated solution at a sufficiently high sample-to-water ratio where a thermodynamic equilibrium exists between the undissolved solute and the dissolved solute. When water is added to such saturated solutions, it is expected that more material will dissolve, ultimately leading to a completely dissolved sample.

However, this strategy is not applicable to polymers because they consist of molecules of different molecular weights, and each weight fraction exhibits its own water solubility characteristics. For example, low-molecular-weight components could be completely in solution, while higher-weight components may be approaching saturation and some other fractions may be completely insoluble.

OECD Test Guideline 105 also suggests a column elution method for materials with a water solubility of less than 10 mg/L. However, this method is not appropriate for polymers because of their wide range of molecular weight distribution, which can result in a range of water solubility results being generated. Therefore, the New Substances Program (NSP) does not recommend using OECD Test Guideline 105 to address the water extractability requirement for polymers.

OECD Test Guideline 120

OECD Test Guideline 120 is a solution/extraction behaviour test that is a modified version of the shake flask method from OECD Test Guideline 105 and is considered by the NSP to be more appropriate for polymers. OECD Test Guideline 120 takes into account that polymers consist of components of different molecular weights and that each component exhibits its own solubility characteristics, i.e., the fraction that can be extracted into the aqueous medium. Extractability is an important issue for environmental and human health risk assessments because the water-available fraction is likely to represent those components of the polymer that are of greatest interest due to their bioavailability.

As will be discussed in Section 4, the amount of extractable material depends on sample-to-water ratio, the temperature of the solvent, pH, particle size and residence time of mixing. The guideline has sample preparation steps and methods that address these parameters and provide advice on acceptable conditions for testing.

OECD Test Guideline 120 is an internationally accepted method that can be applied to most polymers. Consequently, the NSP has recommended OECD Test Guideline 120 as the replacement for OECD Test Guideline 105 to test water extractability of polymers.

Although OECD Test Guideline 120 states that the method is not applicable to liquid polymers or those that appear as liquids due to impurities like solvents, it is recommended that water extractability be investigated using this guideline regardless of the physical state of the polymer. Guidance on sample preparation in these cases (not provided in OECD Test Guideline 120) can be found in Section 5 of this document.

In the case of polymers for which testing under OECD Test Guideline 120 is not amenable, a waiver for water extractability should be requested (see Section 7).

4. Behaviour of Polymers in Water

Dissolution of Polymers

Ideally, an aqueous polymer solution would be described as a uniform distribution of a macromolecular solute in water. In practice, an aqueous polymer system is not necessarily representative of the initial polymer, because water can preferentially extract some molecules, leaving others remaining within the polymer bulk.

Dissolution of polymers generally involves the following steps:

a) Permeation/diffusion of water molecules into the polymer bulk:

- The process of dissolving a polymer begins with water molecules permeating its surface.

b) Swelling of the polymer bulk:

- The swelling starts at the surface and, if water molecules are able to penetrate further, continues into the bulk of the polymer.

c) Detachment of molecules from the polymer bulk:

- During the process of swelling, some low-molecular-weight molecules may detach from the bulk and diffuse into the water phase.
- However, if the polymer is cross-linked, the swelling may only result in a gel formation.

Factors Affecting Water Extractability

The following factors can affect water extractability during testing:

a) Polymer-specific properties:

- ***Polymer structure:*** The amount and type of hydrophilic groups and their distribution.
- ***Molecular weight:*** The general observation is that the greater the molecular weight of a particular polymer, the lower its solubility.

b) Test conditions:

- ***Sample preparation:*** Methods to separate the polymer from a mixture or isolate it from a solvent must maintain the integrity of the polymeric substance with respect to its molecular weight distribution as well as its water extractability.
- ***Surface area of the sample:*** Because dissolution predominantly occurs at the surface of the polymer bulk, samples with high surface area (i.e., composed of small particles) dissolve faster.
- ***Water volume-to-sample mass ratio:*** Using an insufficient amount of water may underestimate extractability results.
- ***Rate of mixing:*** Stirring replenishes the solvent near the surface of the solute.
- ***Temperature:*** Heating the solution imparts a higher kinetic energy to solvent molecules. Heat may also be generated during water/solute interactions (e.g., hydrogen bonding), which in turn can facilitate dissolution.
- ***Water quality:*** Some polymers that are not readily available in water may dissolve in aqueous salt solutions ("salting-in" effect). In other cases, salts may reduce the hydration of polymer molecules ("salting-out" effect).

- ***pH:*** Polymers with ionizable moieties, such as carboxylic acid groups or amine groups, can be rendered more water-available in aqueous solutions by the addition of a base or acid, respectively.
- ***Work-up:*** The use of high-speed centrifugation or very fine filters may separate extracted material from otherwise stable water dispersion.

5. Technical Guidance for Applying OECD Test Guideline 120

The water extractability of polymers is affected by many factors. Comparable results can only be obtained using consistent test conditions and procedures. OECD Test Guideline 120 offers some standard parameters for the following factors:

1. Particle size between 0.125 and 0.25 mm
2. Sample-to-water ratio of 10 g per 1 L
3. Temperature of 20°C
4. Mixing time of 24 hours

However, the method does not specify parameters for the following:

1. Quality of water
2. Rate of stirring
3. Centrifugation speed and/or filter size during work-up

It is recommended by the NSP that the water extractability of polymers be determined using OECD Test Guideline 120 with the following guidance:

- The water should be distilled or deionized.
- As prescribed by the Regulations, the pH of the aqueous phase should be 2, 7 or 9 respectively, prior to adding the notified polymer. The pH should be adjusted with hydrochloric acid or sodium/potassium hydroxide to avoid the use or formation of a buffer system.
- The sample-to-water ratio should be 10 to 1000 (grams of polymer to millilitres of water).
- The sample should be agitated for 24 hours at 20°C. Standard laboratory shakers are deemed sufficient to mimic the environmental action of water.
- Although some liquid polymers may not be amenable to testing, the formation of stable liquid dispersions (i.e., emulsions) should be investigated. Stable emulsions are considered by the NSP to be water-available.
- Polymers in solvents should be dried appropriately so that the integrity of the polymer is not affected. For example, heating in an oven to remove residual solvent can result in the loss of oligomers and promote additional polymerization of the substance. Such pre-treatment would be considered by the NSP to invalidate the water extractability results. Rather than attempting to remove all the residual

solvent, any remaining solvent can be analyzed along with the polymer sample and that amount be excluded from the water extractability results.

- While OECD Test Guideline 120 suggests filtration or centrifugation to achieve a **clear aqueous phase**, the NSP is seeking to quantify the entire bioavailable portion of the polymer, which in certain cases could include a stable dispersion or emulsion in water. Therefore, one of the following techniques may be considered when removing suspended material (please read "Note" under Analysis and Reporting):
  - Low-speed centrifugation: Considered ideal if conducted over a reasonable time frame (typically for two hours or less) using speeds below ultra centrifugation.
  - Filtration: Use a filter that does not plug or require excessive pressure (filter sizes that are too small may result in separation of larger-molecular-weight fractions and/or polymer degradation by shear forces in the filter). If clogging occurs, the resulting filtrate will not be representative of the polymer's water availability. Clogging significantly reduces filter pore size, thus invalidating the water extractability result. If the filters clog, centrifugation should be used.

Analysis and Reporting

OECD Test Guideline 120 refers only to a suitable method of analysis for determining the extractable components and suggests different methods for performing this analysis. From the perspective of the NSP, aqueous gel permeation chromatography (GPC) would be the preferred method of analysis, as it allows correlation between molecular weight and water availability, thereby allowing differentiation between extractability of unreacted monomers and additives or impurities.

Results should be reported as % and calculated as follows:

Amount of substance extracted (mg) x 100% =
Original sample weight (mg)

Reminder: the sample-to-water ratio should be 10 to 1000 (grams of polymer to millilitres of water).

This reflects a relative percentage of water availability that includes solubilized as well as dispersed components. The result of this water extractability test may therefore not be used to calculate an octanol-water partition coefficient (K_{ow}).

The New Substances Program requests detailed reports, including all pertinent information on conditions of test performance and observations.

Note: As the name implies, the OECD Test Guideline series provides guidelines on how to perform certain tests. Specific test procedures or conditions may have to be adjusted according to the specific properties of the substance to be tested. The New Substances Program, in reviewing information submitted under the Regulations, may not find studies acceptable where performance and/or conditions resulted in interference that affected the study outcomes.

6. Surface-active and/or Water-dispersed Polymers

In some cases, surface-active polymers can form colloidal dispersions (solid polymers) or emulsions (liquid polymers). The size of the dispersed particles is generally between 1 nm and 1 μm in diameter. Although some colloidal dispersions are inherently stable, polymer dispersions often need to be stabilized.

Providing water extractability data is not necessary for surface-active polymers and polymers formulated in water and marketed as such, since they will be assumed to be completely water-available.

Reporting requirements are addressed in the next section.

7. Water Extractability Considerations

Reporting Requirements

Polymers with less than 2% water extractability are generally considered of low bioavailability in the aquatic environment, which is a factor in determining the risk the substance may pose to human health and the Canadian environment. Accordingly, a threshold of 2% water extractability was introduced into the Regulations. Polymers having a water extractability of 2% or less are exempt from the ecotoxicity, biodegradation and hydrolysis information requirements prescribed under schedules 10 and 11.

- **Completely water-available polymers**

Polymers marketed in the form of emulsions or dispersions and those that are capable of forming stable emulsions or dispersions are regarded as "100% water-available." Therefore, providing water extractability information is not necessary. However, it must be clearly indicated in the NSN reporting form that the polymer is 100% water-available. Ecotoxicity, biodegradation and hydrolysis test information continue to be required as set out in the Regulations.

- **Water-available polymers**

For polymers that are not completely water-available, water extractability has to be determined. Ecotoxicity, biodegradation and hydrolysis test information continue to be required as set out in the Regulations.

- **Polymers not available in water**

Polymers with demonstrated water extractability of less than or equal to 2% are not considered to be water-available and, as set out in the Regulations, ecotoxicity, biodegradation and hydrolysis information are not required.

Surrogate Substances

Based on the complexity of polymer solubilization in water, it is generally difficult to find viable surrogate substances. The New Substances Program recommends that notifiers submit water extractability data on the notified polymer rather than surrogate data. However, if submitting surrogate data, they must be supported by the following information:

Size

Number average molecular weight (Mn) and oligomeric content

Composition and structure

Monomer composition including weight percent
Final structure

Additional information

Use

Presentation of information on a surrogate

Except for the structure, information on the surrogate substance should be provided, preferably in a tabular format, comparing the above parameters of the notified substance and surrogate.

Waivers

If a polymer is considered completely water-available, a statement to this effect is sufficient to address the water extractability requirement and a waiver request is not required. If surrogate or alternate data are provided, a waiver request is not necessary either.

There are limited circumstances in which waivers would be granted. When it is technically unfeasible to determine the water extractability, the notifier should request a waiver under paragraph 81(8)(*c*) of the Act. If a waiver is granted, the extractability remains unknown; consequently, the substance will be subject to the ecotoxicity, biodegradation and hydrolysis requirements as set out in the Regulations.

Notes

- As previously indicated, the result of a water extractability test cannot be used to calculate the *n*-octanol/water partition coefficient (K_{ow}).
- While OECD Test Guideline 120 provides guidance on test performance, not all parameters affecting the test results are specified in detail; consequently these details may need to be chosen based on a polymer's properties. Environment Canada and Health Canada review all information submitted and may not find studies acceptable where performance or conditions resulted in interference that affected the outcomes.
- The acceptability of surrogate data or alternate data and waiver requests will be evaluated by scientists of the NSP on a case-by-case basis. If it is determined that the surrogate or alternate data is not acceptable, the notification would be considered incomplete.
- Pre-notification consultations (PNCs) are recommended when an opinion on the acceptability of test conditions is sought or when guidance is needed on the acceptability of surrogate data and/or waiver requests.

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For additional information or documentation regarding the *New Substances Notification Regulations*, please visit the New Substances website at:
www.ec.gc.ca/substances/nsb/eng/index_e.htm

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