



Watson's Notes

Innovative Solutions
for difficult problems

"You have recently been in Afghanistan, I perceive"

With these words was born the most famous team in detective fiction; Sherlock Holmes and his trusted comrade and biographer, Dr. John H. Watson.

In the spirit of Watson, who chronicled the exploits of Holmes, we have created this newsletter named "Watson's Notes".

In the pages of "Watson's Notes", modern day scribes document the discoveries, unusual cases and other news of Investigative Science Incorporated, our scientific consulting firm in Burlington, Ontario, Canada.

Please contact us if you have comments, and please read on.

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INSIDE

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New Substance Notification (NSN) Testing Requirements under the Canadian Environmental Protection Act (CEPA)

Overview of the OECD methods required by CEPA

Canadian legislators require that testing offered in support of regulatory applications, such as the introduction of new chemicals into Canada (New Substance Notification Regulations, see Canada Gazette Part II, Vol 139, No. 19, pg 1864-1928) must be done according to the Organization of Economic Cooperation and Development (OECD) methods. These OECD methods are used worldwide for chemical registration and are similar to the OPPTS tests used in the United States. We also provide these testing services in compliance with the Good Laboratory Practice (GLP) for European, Australian and other jurisdictions worldwide and "in the spirit of GLP" for Canadian work. The OECD is an international organization, of which Canada is a member.

This series of OECD methods are analytical tests designed to assess the chemical, physical and environmental properties of chemicals and polymers. The methods themselves are contained in a document entitled the "OECD Guidelines for Testing of Chemicals, Section 1, Physical-Chemical Properties". There is also a Section 2 entitled: "Effects on Biotic Systems". These tests are largely focused on the behaviour of chemicals in the environment and describe a range of bioassays. Section 3 covers "Degradation and Accumulation", while Section 4 deals with "Health Effects". The latter are largely toxicity tests.

The physical and chemical tests described in Section 1 are the focus of our work at ISI. These tests include:

OECD 101	UV-Vis Absorption Spectra
OECD 102	Melting Point
OECD 103	Boiling Point
OECD 104	Vapour Pressure
OECD 105	Water Solubility

OECD 106	Adsorption-Desorption (to and from representative soils)
OECD 107	Octanol-Water Partition Coefficient (Flask Method)
OECD 108	Complex Formation Ability In Water
OECD 109	Density
OECD 110	Particle Size
OECD 111	Hydrolysis as a Function of pH
OECD 112	Dissociation Constants in Water
OECD 113	Screening Test for Thermal Stability and Stability in Air
OECD 114	Viscosity of Liquids
OECD 115	Surface Tension
OECD 116	Fat Solubility
OECD 117	Octanol-Water Partition Coefficient (HPLC Method)
OECD 118	Molecular Weight by Gel Permeation Chromatography (GPC)
OECD 119	Determination of Low Molecular Weight Content by GPC
OECD 120	Solution/Extraction Behaviour of Polymers in Water
OECD 121	Estimation of the Adsorption Coefficient on Soil and Sewage sludge by HPLC
OECD 122	Partition Coefficient, pH-Metric Method for Ionisable Substances

When are these methods used?

In practice, the CEPA regulation for New Substance Notification testing, which was released in October 2005, only requires a subset of this list for regulatory submission.

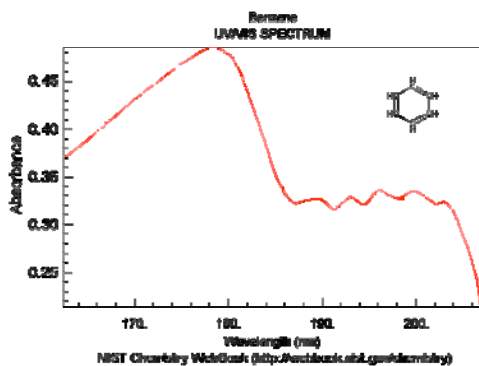


Figure 1 - UV Spectrum

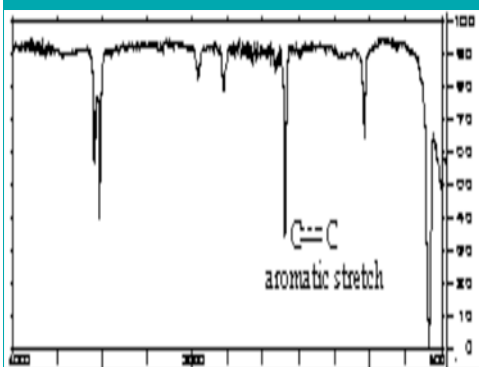


Figure 2 - IR Spectrum

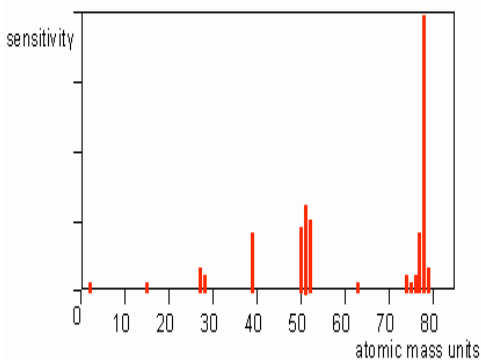


Figure 3 - Mass Spectrum

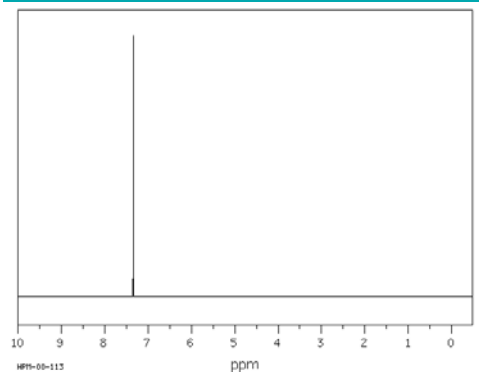


Figure 4 - NMR Spectrum

Which test is required depends on how much material is produced, whether the new material is a chemical (typically discrete molecular weight, no repeat units) or polymer (contains repeat units and a distribution of molecular weights), as well as other factors.

For example, new chemical testing may typically include:

1. Melting Point (OECD 102)
2. Boiling Point (OECD 103)
3. Density (OECD 109)
4. Vapour Pressure (OECD 104)
5. Water Solubility (OECD 105)
6. Octanol/Water Partition Coefficient (OECD 117)
7. UV, IR, Mass or NMR Spectrum (OECD 101)
8. Adsorption-Desorption (OECD 106), for chemicals produced in amounts greater than 10 000 kg and having a water solubility more than 200 ug/L
9. Hydrolysis (OECD 111) for chemicals produced in amounts greater than 10 000 kg and having a water solubility more than 200 ug/L

For polymers, the following may typically be required:

1. Water Extractability (OECD 120)
2. Octanol-Water Partition Coefficient (OECD 117)
3. Number Average Molecular Weight (OECD 118)
4. Concentrations of material having molecular weights of less than 500 and 1000 Daltons (OECD 119)

How do the Methods Work?

UV, IR, Mass or NMR Spectra (Method 101)

Method 101 actually only deals with UV spectra but the CEPA regulation allows any of the above spectral techniques to be used. Examples of the four types of spectra for benzene are shown in Figures 1-4. The spectrum of a substance refers to a characteristic behaviour, usually the absorbance of radiation, displayed by the substance when placed in the appropriate device.

The UV spectrum is a characteristic absorption of ultraviolet light caused by specific chemical groups in the molecule. The printout from the UV spectrophotometer is called a spectrum. The IR spectrum is similar but uses infrared light. Both types of spectra provide general information about the chemical groups present in the new substance.

Both mass and NMR spectra are more complex than UV or IR and yield specific and useful structural information about the molecule. For regulatory purposes the key point is that all four of these techniques give a spectrum that is characteristic of the substance in question. They may therefore be used to verify the identity of the material and, in some circumstances, may verify the purity or show the presence of impurities, starting materials, and other reactants.

Melting Point, Boiling Point, and Density (Methods 102, 103, and 109)

These tests are relatively straightforward physical tests used to characterize the new substance. The melting point is determined using a melting point apparatus. Crystals of the substance are heated in a controlled fashion until they melt. In a standard automatic melting point apparatus, the melting point is determined when a light beam is able to pass through the sample.

The boiling point is determined in a similar fashion. Traditionally, the substance is placed in a glass vessel along side a thermometer and heated in a hot oil bath until it boils.

Density is simply the mass of the substance that can be held in a container of a known volume. The bulk density is most commonly used for regulatory purposes. Bulk density does not account for the air spaces between the particles of the sample. As such, it gives an estimate of the volume occupied by a known amount of the sample. As an illustration, a kilogram of Styrofoam chips used for packing may fill a small room. A kilogram of gold is only slightly bigger than a pile of business cards one centimetre high.

Vapour Pressure (Method 104)

Vapour pressure is an indicator, along with melting point, of the volatility of a substance. The test measures the amount of substance that is released into a stream of inert gas, which passes over the material at a defined flow rate and temperature.

Vapour pressure is often estimated based on the melting and boiling points. In our lab, this parameter is actually measured as described in Method 104.

The Solubility Tests: Water Solubility and Water Extractability (Methods 105 and 120)

The solubility tests, simply put, measure the amount of substance that dissolves in water, buffer or other solvent. There are actually a series of similar tests designed to measure solubility in fat (Method 116), octanol/water mixtures (Method 107) and water (Method 105) for pure chemicals and Method 120 for polymers. Method 105 may also be used to determine octanol solubility.

The tests are similar in concept but there are subtle differences in experimental set-up and interpretation. For example, Method 105 is used to determine the solubility of pure chemicals in water. The test substance is stirred or shaken in a flask with water at a carefully controlled temperature until the concentration of the substance in the water has stabilized. This may take several days to a week.

For polymers, using Method 120, the water must be buffered to neutral, acidic or basic pH depending on the type of polymer. The required pHs are specified in the CEPA regulation.

All of the solubility methods require a specific method of detecting the chemical of interest in water, octanol, fat or whatever the solvent happens to be. This must be a reasonably specific test, usually requiring a specific UV or IR absorbance, a specific chemical reaction, or a chromatographic separation or, in some cases, all three. Non-specific tests such as weight (gravimetric) or Total Organic Carbon (TOC) are frowned upon in the method descriptions. They are typically used only when there is no other workable option.

Octanol/Water Partition Coefficient by HPLC or Flask Method (Methods 117 or 107)

The octanol/water partition coefficient is simply the extent to which a substance partitions between octanol and water. Octanol floats on the water and if the substance is fat soluble, it will partition preferentially into the upper, or octanol, phase. The partition coefficient (P_{ow}) is the ratio of the concentration of the substance in the octanol phase divided by the concentration in the water phase.

The test was developed many years ago to estimate the extent to which a chemical dissolved in water would partition into a cell membrane, such as the skin of a mammal. Octanol was used because its polarity was thought to be closely similar to that of the inside of a cell membrane.

The test presents a fairly simplistic view of a chemical transport, but the octanol/water partition coefficient or its logarithm ($\log P_{ow}$), has gained worldwide acceptance in the environment regulatory world. It provides a useable estimate of the tendency of a material to bioaccumulate in animals. According to the theory, any chemical with a $\log P_{ow}$ of 3 or greater will bioaccumulate by building up in animal fat, assuming that the chemical is not readily broken down. A value of 3 means essentially that the material is 1000 times more soluble in octanol (and theoretically, fat) than in water. It has been shown experimentally that there is a good correlation between $\log P_{ow}$ and bioaccumulation in fish.

The original method (107) employed a flask containing octanol, water and the test substance. More recently, High Pressure Liquid Chromatography (HPLC) using a C18 reverse phase column (Method 117) has become the preferred method, according to CEPA. This approach exploits the correlation between $\log P_{ow}$ and HPLC retention time (how long it takes for the unknown compound to elute from the HPLC). Using Method 117, the HPLC is calibrated using a series of chemicals with known P_{ow} . A plot of $\log P_{ow}$ versus the log of the retention time gives a straight line (see Figure 5). The unknown is run on the same system, and from the retention time, the P_{ow} can be determined.

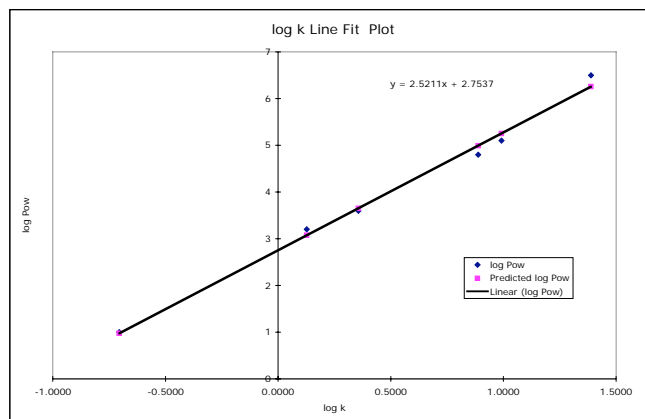


Figure 5 - $\log P_{ow}$ versus $\log k$

In practice, these methods have some limitations. For example, many factors such as charge affect the HPLC retention time of a compound. For this reason, the HPLC method is usually restricted to neutral or weakly acidic or basic compounds. For ionisable compounds the potentiometric method (Method 122) is gaining acceptance. We'll discuss that in a future issue. As well, with a polymer, one usually sees a series of peaks, so the calculated P_{ow} is the weighted average of the peaks that are detected. Unfortunately, it is usually not possible to know whether everything is being detected. Hence the estimated value may be hugely in error.

**ELEMENTARY
MY
DEAR
WATSON**

Sherlock Holmes and Doctor Watson were doing their usual investigative business one day, when they uncovered an amazing painting.

At first glance, it looked like a picture of normal oak tree, in the middle of a wilderness, but if you looked closer, you could see that it was a very surreal painting: The tree's trunk was actually made of fire, and it's branches were made of ice, clouds and earth.

"What is it?" asked Watson in awe.

"It's an element tree, my dear Watson," said Holmes

The flask method is reportedly useful for compounds with a log P_{ow} of between -2 and 4 , while the HPLC method is good up to log P_{ow} of 6 . Beyond that we are into the realm of estimation. There are several models available, but their reliability is largely unsubstantiated. The main problem is that there are precious few examples of molecules with a log P_{ow} greater than 6 with which to calibrate the models. In virtually all cases, it is not possible to experimentally verify the estimates once the P_{ow} is greater than about 8 . The models, however, will happily predict log P_{ow} s of 15 or more for some fat soluble molecules.

The key point, however, is whether a molecule has a log P_{ow} of 3 or more, indicating that it may bioaccumulate. It probably doesn't matter whether a molecule has a measured or theoretical value of 6 or 15 . They are both going to be fat soluble.

Soil Adsorption-Desorption (Method 106)

The soil adsorption-desorption test determines how readily the test substance sticks to soil, and is released by the soil. The test employs 5 well characterized local soils having a range of pH values, clay, silt and organic content. The new chemical is dissolved in salt water and incubated with the soils. The amount and rate of disappearance of the chemical from the soil is measured. Once the material has adsorbed to the soil, the rate of release into fresh water is measured. Typically, the regulatory agencies are only interested in the adsorption phase and are satisfied with the screening portion of the test, called Tier 1. They usually don't require an in-depth determination of the kinetics of adsorption and desorption, such as is described in the later Tiers of the most recent version of OECD 106.

Hydrolysis (Method 111)

As the name implies, the hydrolysis test measures the rate at which a chemical breaks down in water at a range of pH values and elevated temperatures. The test requires a detection method capable of distinguishing the starting material from the breakdown products. This typically requires some type of chromatography such as HPLC or gas chromatography. The test generates many samples for analysis over a period up to 30 days. This makes the hydrolysis test one of the most expensive to conduct.

Molecular Weight and Low Molecular Weight Content by Gel Permeation Chromatography (Methods 118 and 119).

Molecular weight determination is required only for polymers and is done by Gel Permeation Chromatography, or GPC according to Method 118. GPC is a version of HPLC that separates molecules by their size, or molecular weight, rather than other physical characteristics.

The test substance usually appears on a GPC chromatogram as one or more broad humps reflecting a distribution of molecular weights. This distribution is a feature of synthetic polymers and is unlike pure chemicals, which have a single discrete molecular weight. To determine the proportion of material at each molecular weight, the hump is sliced electronically at regular intervals, corresponding to known molecular weights. The amount of material in each slice is proportional to the area under the curve of the printout (chromatogram).

The CEPA regulations require that the number average molecular weight (one of many ways to report the average weight) be reported, as well as the percentage of material with molecular weight less than 500 and 1000 Daltons. According to the regulations, polymers having less than 5% of their weight below 1000 Daltons and less than 2% below 500, fall into a lower risk category than those with greater amounts of low molecular weight material.

The low molecular weight content is typically determined by GPC using Method 119. This is closely related to Method 118 but focuses the calibrations in the 500-1000 molecular weight range and, depending on the polymer, may require a different GPC column set up. The absence of low molecular weight material can, in some cases, be demonstrated by the GPC profile obtained by Method 118, however, if the hump extends into the low molecular weight region, Method 119 is needed in order to obtain an accurate percentage.



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