# *Workshop of the Latest Development on the Evaluation Method of PBT (Persistent Bioaccumlative Toxic) Chemicals*

*— Focusing on the Environmental Fate, Bioaccumulation, and Safety Assessment of Cyclic Siloxane —*

*August 29, 2017*

*The Society of Silicon Chemistry Japan*

# PROGRAM

The Symposium was held on 29th August 2017 in Tokyo. It is organized by The Society of Silicon Chemistry of Japan, and co-sponsored by International Council of Chemical Association, American Chemistry Council, The Chemical Society of Japan, Japan Chemical Industry Association, and Silicone Industry Association of Japan.

# Opening Speech

**Mitsuhiro Takarada**
*Vice Chairman, The Society of Silicon Chemistry, Japan (SSCJ)*

About SSCJ
The society was founded in 1996 with the aim of promoting silicon development as well as development in other related chemistries. We currently have 424 members in Japan and abroad. We welcome researchers in silicon chemistry and related industries with an inexpensive membership fee of ¥2,000 (USD19)/y.

SSCJ has the following four missions:

1. Support the growth of young scientists
2. Strengthen cooperation with scientists in Asia and beyond
3. Expand membership and exchange knowledge between various fields
4. Enhance silicon chemistry literacy by various fields

The activities of SSCJ include the following:

- Holding domestic two-day seminars each year
- Holding ASIS symposium once every two years
- Dispatching young members to overseas conferences
- Presenting awards to scientists in academia and industry with outstanding achievements each year

# Overview of Silicon Chemistry, Application/Physical-Chemical Properties

**Ralf Maecker**
*Chair, CES-Silicones Europe / Momentive Performance Materials*

## Introduction

Silicones are specialty products. The backbone of inorganic silicon and oxygen atoms drives the unique properties of this chemistry set which makes them distinctly different from carbon chemistry. Silicones are only used where their special performance is needed. Siloxanes are indispensable, critical building blocks for all silicone products.

## Chemistry and characteristics of Si-based materials

'Silicone' is a generic term referring to a class of synthetic polymers based on a framework of alternating silicon and oxygen (siloxane) bonds with at least one organic group attached to the silicon atom via a direct carbon-silicon bond. The silicone family includes siloxanes and silanes, all of which are widely used in thousands of products. Due to their molecular structure, silicones can be manufactured in many forms such as solids, liquids, semi-viscous pastes, greases, oils, and rubber.

Silicone polymers are typically liquids of varying viscosity that may be linear or branched, but are individual polymer strands. Structuring of the polymer can occur by cross-linking the individual chains. Cross-linking is accomplished by synthesizing polymers with functional groups on the chain that either react with each other or with moisture in the atmosphere. Cross-linked silicone polymers can have very different properties dependent on the degree of cross-linking. Gels are lightly cross-linked and can flow. Foam and rubber silicone features a higher degree of cross-linking and may be reinforced. Hard coats are highly cross-linked and reinforced.

Silicone materials offer a host of useful physically and chemically useful characteristics that include thermal stability; resistance to oxidation, ozone, and UV exposure; low surface tension for good wetting, spreading, and flow; good dielectric properties; water repellency (hydrophobicity); low flammability; high gas permeability; high compressibility and shear resistance; limited organic compatibility; and softness and flexibility. The structure of silicones gives them unique properties critical to demanding applications such as: personal care (antiperspirants and hair conditioners); automotive (coatings, lubricants, ignition cables, and engine gaskets); construction (sealants and adhesives); electronics (smart phones, tablet computers, and keyboards); health care (tubing, syringe coatings, cushioning, catheters, and masks); textiles (coatings for water resistance, improvements for tear strength and abrasion

resistance); as well as renewable energy applications (insulators in solar and wind energy solutions).

**Introduction to cyclic and volatile methyl siloxanes (cVMS)**

Manufacturing of silicones start with silicon metal sourced from natural SiO2 (quartz). Figure 1 describes the main steps in manufacturing silicone.

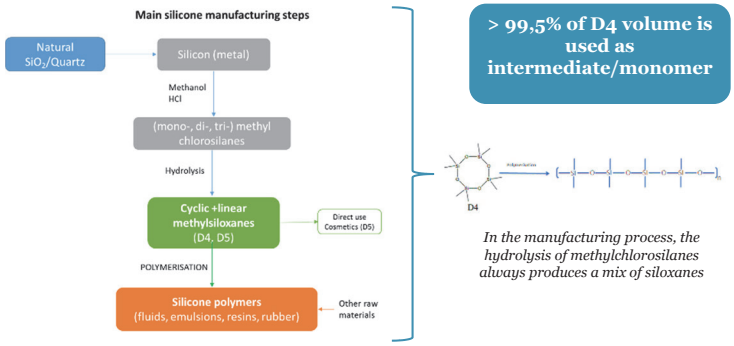
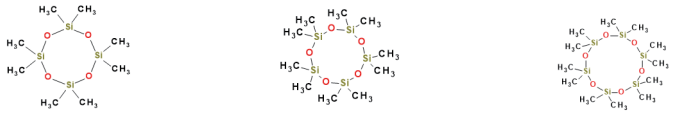



Figure 1: D4 is a vital building block for all silicones

It is important to note that in the manufacturing process the hydrolysis of methylchlorosilanes always produces a mix of siloxanes that are then separated. Once separated, cVMSs can be directly used in products. cVMSs are basic members of the family of silicone materials and are raw materials for the manufacture of silicone polymers. cVMSs are clear, volatile, low molecular weight liquids with very low water solubility with D4, D5, and D6 as examples. Figure 2 shows the chemical structure of these cVMSs.



D4 D5 D6

Figure 2: Chemical structure of cVMSs

**Challenges of cVMS: Silicone chemistry**

The backbone of inorganic silicon and oxygen atoms combined with the organic functionality drives the unusual combination physical and chemical properties including high hydrophobicity and lipophilicity; low water solubility; volatility; large molecular size; very low polarizability; significant hydrogen bond acceptor characteristics; higher bond strength of Si-O bonds compared to Si-C bonds; and undergo biotransformation in both aquatic and terrestrial organisms.

In summary, the industry has invested and investigated the properties and chemistry of silicones

over the past four decades. Much information needs to be looked at in order to understand them. Silicones are very different from organic chemistry.

It is important to note that silicone chemistry is fundamentally different from carbon chemistry. Silicon is one period lower than carbon in the periodic table of elements and silicon has a greater capacity than carbon to share electrons with oxygen. This leads to stronger bonds with higher bond energies, higher bond angles, and shorter than expected bond lengths as compared to the carbon-oxygen bond. The nature of the silicon-oxygen bond makes siloxanes molecules like D4 flexible, which results in weak interactions between other siloxane molecules. This is illustrated by the lower surface tension, viscosity, and vapor pressure of siloxanes compared to hydrocarbons of similar molecular weight. Their large size combined with a moderate ability to accept hydrogen bonds leads to differences in the ability of D4 and other siloxanes to interact as solutes with environmental 'solvents' or environmental media such as water, organic carbon in soil or sediment, and lipids in biota. These characteristics lead to different behavior in the environment as compared to carbon compounds.

**Summary**

Silicones are among the most extensively studied materials used in consumer and industrial applications today with over 1000 studies over more than 35 years. Silicones are extremely versatile with wide-ranging properties that make them key component in thousands of consumer and industrial materials. The beneficial characteristics of silicones enable them to be used in a wide variety of applications and products including in construction, transportation, electronics, manufacturing, engineering, health care, cosmetics, and personal care

# Science of PBT Assessment: Overview of Safety Assessment of cVMS

**Kathleen Plotzke**
*ICCA-LRI Chair, Dow Chemical USA*

## The issue

On a global scale, regulatory agencies are implementing risk management measures to control exposure to chemicals considered to be persistent, bioaccumulative, and toxic (PBT) or very persistent and very bioaccumulative (vPvB). In some jurisdictions these decisions are based solely on screening-level criteria with no risk-based assessment. Restrictions based solely on screening-level criteria without consideration of a weighted evaluation of all scientific evidence will drive promising technologies with promising benefits out of the market, which will then limit innovation and commercial growth.

## Scientific evolution of PBT criteria

The Stockholm Convention is a global treaty that was adopted in 2001 and ratified in 2004 to protect human health and the environment from persistent organic pollutants (POP) and PBT chemicals. Growing concern for these chemicals was the impetus for the classification schemes of POPs/PBTs since they last for long time in the environment (persistent), bioaccumulate in fatty tissue of living organisms and magnify as they move up the food chain, are toxic, and travel long distances far from emission sources.

Criteria for the classification of the POPs were developed in the late 1990s by the Criteria Expert Group for Persistent Organic Pollutants. The criteria were based on the properties of a number of compounds, all of which were subsequently classified as POPs under the Stockholm Convention.
Unfortunately, the criteria used to identify a chemical as PBT is based on the state of the science in the late 1970s and early 1980s. It was assumed to reflect all chemistries and is not always consistent from one jurisdiction to another.

## Protection goals for POPs

The goal for classifying POPs are to ensure that both humans and the environment are protected. Indeed, UNEP in 2001 stresses, "…significant adverse human health and/or environmental effects…"

## ICCA LRI Workshop

The 2016 ICCA LRI Workshop held in Japan focused on new approaches for weight-of-evidence decision making for PBT chemicals and POPs compared to criteria-based approaches. The session focused on front-line approaches to evaluate PBT chemicals and POPs. Discussions included: development of a quantitative and transparent weight of evidence (WoE) approach for characterization of PBT/POPs compared to criteria-based assessments; revision of the guidance on REACH's PBT/vPvB criteria; importance of dietary exposure in evaluating the bioaccumulation of poorly water soluble substances; Multibox-AQUAWEB model for bioaccumulation assessment; the measurement and influence of metabolism in bioaccumulation assessments; challenges in assessing the toxicity of poorly water soluble chemicals; weight of evidence (WoE) approach for assessing literature on concentrations of environmental chemicals in breast milk and formula; and the challenges of assessing persistence in a regulatory context.

What follows are the major findings and recommendations from the workshop. There is a weaknesses of the current numerical POPs/PBT criteria including a lack of realism in existing standardized testing guidance and a lack of understanding/use of realistic exposure potentials for humans and the environment. It is important and opportune time to step back and redefine PBT and POP concerns and the protection goals and design higher tiered standardized testing to protect against false negatives and false positives in achieving this goal. Decisions based solely on numerical criteria without a weighted evaluation and integration of all scientific evidence could drive technologies with promising benefits out of the market, which would then limit innovation and commercial growth and could miss chemicals of real concern. The chemical industry is ideally positioned to collaborate and develop partnerships to develop specific proposals for enhancing the realism of PBT/POP assessments globally and share best practices for acceptance of new methods and approaches. ICCA-LRI can help develop new methods for assessment of substances that may exhibit PBT/POP properties under real life conditions by working collaboratively across sectors with academics and regulators. ICCA-LRI can catalyze the education of the next generation of experts and also serve as a forum for transfer of information on these new methodologies/best practices especially for emerging countries.

## Key science for cVMS PBT/POP assessments

Current environmental screening criteria predict that some cVMS have PBT or POP properties. Research shows that information beyond screening criteria must be evaluated to understand cVMS behavior in the environment. A robust quantitative WoE assessment conducted by Academic experts concluded that all lines of evidence shows that the cVMSs have very different, physical, chemical, and biological properties from the legacy POPs. Refined approaches are needed, and when applied these show that these materials should not be classified as P, B, or T or as vP or vB.

Some jurisdictions only look at screening-level criteria. Not looking at the overall science could prevent the development of important technologies. The criteria for determining which chemicals are PBT or persistent organic pollutants (POP) are to prevent damage (adverse effects) from them to human health or the environment. Unfortunately, the criteria for determining a PBT was based on the science of the 1970s and were assumed to reflect all

chemistries, which is not appropriate for many of the new chemistries today. .

Regarding the key science of persistence, it is important to look at both laboratory and field data on cVMS to fully understand persistence of cVMS in the environment. Many chemicals partition into multiple environmental compartments, but persistence in the major compartment or final sink is most appropriate for assessing persistence in the global context. The overall persistence (POV) is more important for the cVMSs than for other classes of chemicals, such as the classical POPs. Since the cVMSs partition readily to atmosphere (major compartment) where they are degraded more rapidly than in other matrices, their presence in the global environment is much shorter (months) than the classical POPs where global lifetimes are much longer (several years). Due to the physical properties of the cVMSs, traditional laboratory tests for persistence even in sealed systems are not appropriate for extrapolation to the environment because they do not consider rapid partitioning to air, which is the final sink in the environment.

Figure 1 shows how cVMSs are exchanged between media and how they react in the environment.

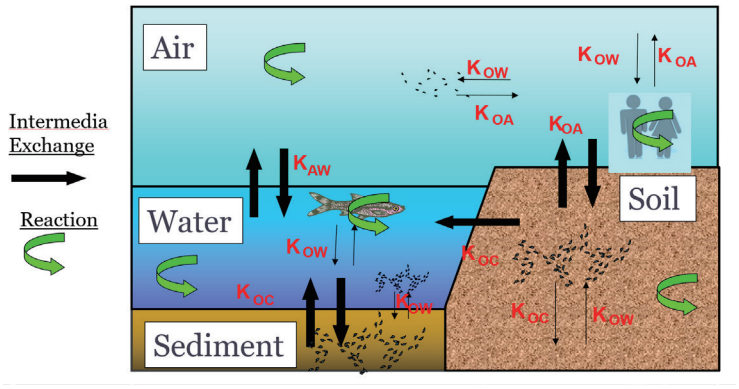



Figure 1: Understanding cVMS Persistence

Regarding bioaccumulation concerns in the food web, it is important to remember that substance must accumulate or biomagnify sufficiently to potentially cause significant adverse effects in populations and ultimately affect ecosystems. An example would be DDT metabolites that affected raptor populations in the USA. When these criteria were put into place, the intent was to prevent significant and adverse effects on human health and the environment. However the criteria were only intended to screen for problem substances and then use current science to determine their actual impacts. The question is how to best predict biomagnification, which is a complex ecosystem phenomenon that is difficult to model in the laboratory taking into consideration the intrinsic properties of substances being evaluated. Bioconcentration factor (BCF) is typically used as a surrogate for biomagnification.

Regarding the evolution of PBT criteria for bioaccumulation, when assessing bioaccumulation potential it is important to consider the intrinsic properties of chemicals for which the criteria were derived, i.e. organochlorines. $K_{OW}$, BCF, and BAF criteria were developed as screening tools with the intention to identify biomagnifying substances. However, since these are proxies, they do not apply to all chemicals equally and vary as a result of the unique intrinsic

properties of chemical substances relative to properties of organochlorines.

Figure 2 shows how bioconcentration is typically used in regulatory assessment.

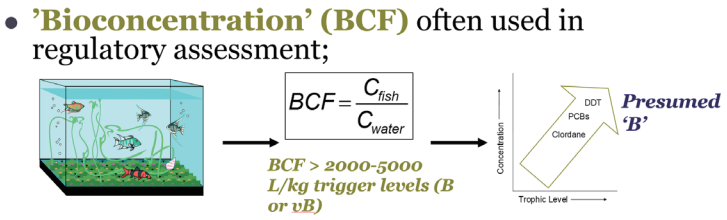



Figure 2: BCF Background

However, the behavior of cVMS in natural systems is very different from laboratory water exposure testing. Indeed, the standard laboratory BCF tests fail to accurately predict the ability of a highly lipophilic, poorly water soluble, and metabolizing substance such as D4, D5, or D6 to biomagnify in the environment. Dietary exposure is the main route of uptake for bioaccumulation of highly lipophilic substances. It is important to understand the behavior of D4 and other cVMSs following dietary exposure for a better comparison to what will actually happen in the field. Several recent field studies from locations in Europe, North America, and Asia have shown that cVMS D4 biodilutes as you move up the food chain. For D5 and D6 there is a lack of full agreement of the TMF across various study areas and this may reflect differences in TMF study design. TMFs may also be biased because of environmental conditions and sample collection. There is also a lack of common sampling areas for the species considered in the TMF calculation and the presence of point sources such as waste water treatment plants can cause concentration gradients in the sampling area. These gradients can have a significant impact on study outcomes, as shown by Gobas et al. in 2014 and 2016. Additional work is currently underway with external experts to validate a Multibox-AQUAWEB model that takes into consideration these variables and confounding factors.

Figure 3 shows D4 TMF behavior in aquatic and terrestrial food webs at Lake Pepin in the USA show that it does not bioaccumulate, in contrast to the results for PCB 180.

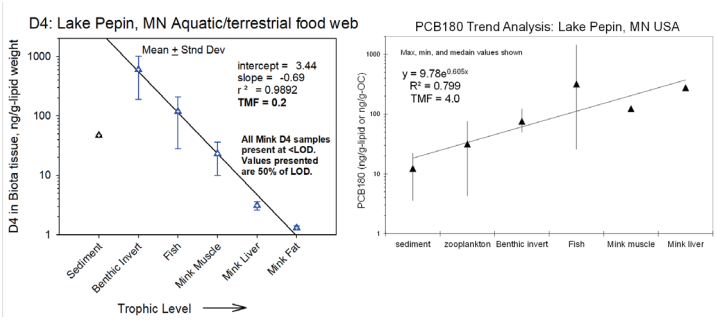



Figure 3: D4 TMF Behavior: Aquatic/Terrestrial Food Web: Lake Pepin (USA) example

Trophic food web biodilution behavior of D4 has been documented in systems varying from freshwater to marine and pelagic to benthicpelagic in food web structure, and reported D4

TMV values are generally less than one, as shown in figure 4.

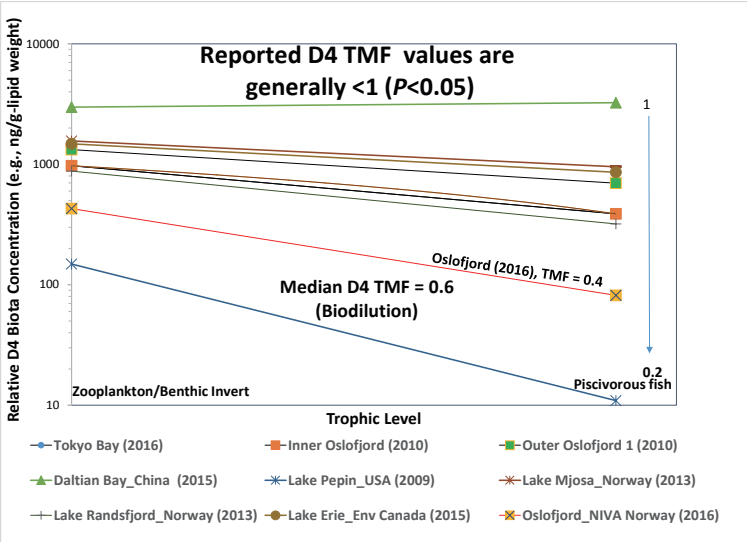



Figure 4: D4 Trophic Biodilution Behavior Observed – new data available from Oslofjord Norway

Two new publications indicate 1) there was no evidence from any of the regression models to suggest biomagnification of cVMS in Tokyo Bay. Rather, the regression models indicated that trophic dilution of cVMS, not trophic magnification, occurred across the sampled food web and 2) . there was no evidence to suggest biomagnification of cVMSs in Oslofjord. Rather, results indicated that trophic dilution of cVMS, not trophic magnification, occurred across the sampled food webs.

**Key science on LRT and back deposition**

The ultimate goal of the POP convention is to protect humans and the environment in local and distant or remote locations from adverse outcomes. LRT and deposition of a chemical to a receiving environment in a remote region is a complex interaction, and this is especially true for substances with unique physical-chemical properties such as cVMSs.

Since half-lives based only on laboratory studies are predicted to be greater than two days, cVMSs may have the potential to undergo long-range transport to remote regions via the atmosphere. However, they are readily degraded in air. It is well established that cVMSs degrade by interaction with OH radicals in the air. cVMSs are mainly released from the urban centers where the OH radical concentrations are much higher than the global average OH radical concentration used to estimate their current half-lives. Very recent work using actual monitoring data demonstrates the real life degradation of VMS in air may be much faster (between 1.7 and 2.7 days) and involve other mechanisms beyond OH radical degradation than what is currently estimated. Discussions with experts from Norway, Stockholm University, and Canada at the SETAC conference provided support for this hypothesis and all expressed an interest to collaborate. Work is underway with external partners to better understand this uncertainty on air half-life.

Although D4, D5 and D6 have been reported in Arctic air, the concentrations are much lower

than in source regions. For example the measured cVMS (D5 and D6) concentrations in the Arctic air are hundreds of times lower than those in the source region, and D4 detection in remote air may be due to analytical artefact. From a report by NILU in 2016, they indicated that "D4 data should be treated with caution as the measured concentrations of D4 might have been affected by degradation effects during storage as shown by Kierkegaard et al. (2013). Thus no firm conclusions can be made regarding the presence of D4 in Arctic air nor any trends in concentration based on findings in this monitoring study."

Very little airborne cVMS will deposit from air to surface compartments in the environment due to their unique combination of partition coefficients. Thus airborne VMS in the remote part of the atmosphere will result in little exposure to surface media. No detections for cVMS in surface media when there is no local release confirmed their low deposition potential. The presence of cVMS does not represent any real exposure risk to biota/humans in remote regions. Therefore, there no potential for adverse outcomes.

**Summary of cVMS WoE assessment: PBT**

Combining all of these lines of evidence shows that the cVMSs have very different, physical, chemical, and biological properties from the legacy POPs. The traditional criteria of persistence, biomagnification used to classify the legacy POPs are not suitable for the cVMSs. Overall, the QWoE analysis shows that there is moderate to strong evidence of no adverse effects from concentrations of D4, D5, and D6 as measured or expected to be in the environment.

In conclusion, refined approaches are needed, and when applied show that cVMSs should not be classified as P, B, or T or as vP, or vB and will not back deposit.

# Dietary Exposure Bioaccumulation Fish Test to Evaluate Bioaccumulation Potential of Chemical: Current Progress in Japan

**Naoki Hashizume**
*Chemicals Evaluation and Research Institute (CERI), Japan*

CERI is one of the biggest laboratories in Japan that conducts the chemicals safety assessments with 40 years of experience in bioaccumulation assessment. CERI has provided the bioconcentration study data for more than 3000 chemicals and has contributed to the progress of bioaccumulation assessments.

**Bioaccumulation assessment in Japan**

Bioaccumulation assessment of chemicals is an important issue for chemical management. The bioaccumulation of chemicals has been assessed since the Chemical Substances Control Law (CSCL) was enacted in 1973. Bioaccumulation assessment is required for chemicals when production or import volume is higher than one ton per year and BCF is used as endpoint. Bioaccmulation criteria in the CSCL state that a chemical is highly bioaccumulative if BCF is greater than 5000 L/kg, and not highly bioaccumulative if log $P_{OW}$ is less than 3.5 or BCF is less than 1000 L/kg.

Regarding the dietary exposure test, in 2004 CERI starting examining the test. In 2010 the OECD conducted the ring test for dietary exposure test and in 2012 the dietary exposure test was included during a revision of the OECD guidelines. In 2013 Japan conducted the ring test for dietary exposure test. In 2016 a committee was formed to discuss the dietary exposure test in CSCL. This year or in 2018 the CSCL will be revised to include the dietary exposure test.

Biomagnification is the increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the food. This is defined in OECD TG 305. In fish the BMF is calculated by dividing the dietary uptake by the sum of gills elimination, fecal egestion, metabolic biotransformation, and growth dilution.

Figure 1 details the dietary exposure test according to OECD TG 305.

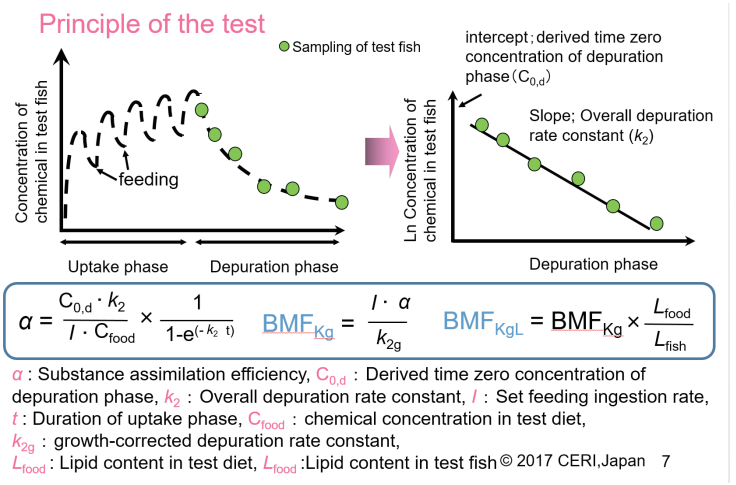



Figure 1: Dietary exposure test (OECD TG 305)

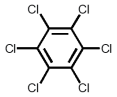
Advantages of the new dietary exposure bioaccumulation fish test include that it is suitable for testing poorly water-soluble chemicals, which may not be technically feasible for an aqueous exposure test. In addition, BMF is a meaningful measure of the potential for the chemicals to undergo dietary biomagnification in the environment.

**Ring test in Japan**

Japan's Ministry of Economy, Trade and Industry (METI) conducted the ring test for the dietary exposure test to fill the data gap for using the results of dietary exposure tests for regulatory purposes. Six GLP laboratories in Japan were selected to perform the test on common carp (*Cyprinus carpio*) using hexachlorobenzene (HCB) in the test diet routinely used in each laboratory. Results showed that $BMF_{KgL}$ and $BMF_{Kg}$ for HCB were below one in all laboratories, that $BMF_{KgL}$ varied more than 10 times between the laboratories, and that $BMF_{Kg}$ varied approximately three times between the laboratories.

Challenges for the dietary exposure test include the fact that the result of ring tests in Japan indicate that $BMF_{KgL}$ may differ depending on test conditions such as the test diet. Therefore, it was decided to conduct the dietary exposure test in different test conditions to investigate the influence of the test conditions on BMF values. Four different feeds were used differing in size and protein/lipid/phosphorus content (Figure 2).

<Test chemical>
Hexachlorobenzene (HCB)



<Diet>

| Name | 2C | Ranchu (Ran) | Otohime (Oto) | Finfish Starter (FF) |
|---|---|---|---|---|
| Particle size | 0.4～0.9 mm | 1.3～1.5 mm | 1.3 mm | 0.8 mm |
| Ingredients | Protein ≥43.0% Lipid content ≥ 3.0% Phosphorus ≥1.5% | Protein ≥53% Lipid content ≥ 12% Phosphorus ≥ 1.0% | Protein 48.0% Lipid content 13.0% Phosphorus 2.0% | Protein ≥55.0% Lipid content ≥ 15.0% Phosphorus ≥ 1.3% |
| Supplier | Nippon Formula Feed Manufacturing. | KYORIN Cooperation. | Marubeni Nisshin Feed Co.,Ltd. | Zeigler Bros., Inc. |

Figure 2: Influence of test conditions

In addition to these differences, the medium was also different with acetone being used in two tests, fish oil at 10% in three tests, fish oil at under 1% in three tests, and corn oil at 10% in one test. This also created differences in the lipid content with the acetone medium tests being at 5.67% and 5.23%, while in the other tests the lipid content varied between 13.9% and 17.1%. There were also different feeding rates with two tests at two and the remaining tests at three. Test condition may influence the $BMF_{KgL}$ values of HCB, and correcting the $BMF_{Kg}$ values by lipid content in the test diet might lead to misunderstanding the results. After normalizing lipid content at 5%, the results still showed a difference of approximately double, but this was less variation than for $BMF_{KgL}$ (Figure 3).

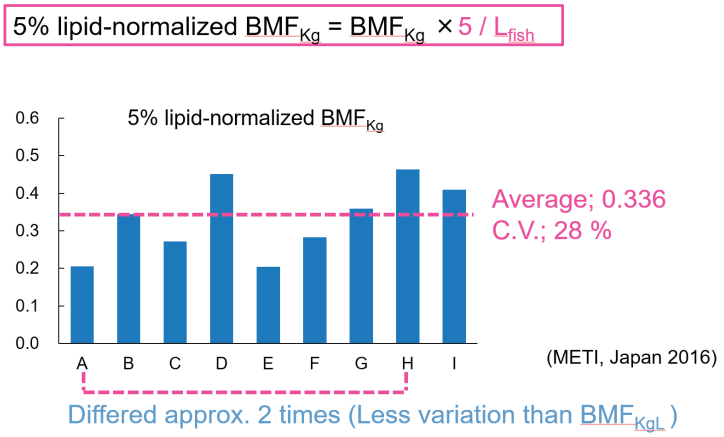



Figure 3: Influence of test conditions

**Dietary exposure test in Japan's CSCL**

METI held a committee to discuss the dietary exposure test in Japan's CSCL. Major comments from experts included that the dietary exposure test is useful for poorly water soluble chemicals, and there is difficulty in conducting the aqueous exposure test. Expert comments also indicated that it was too early to include the dietary exposure test to evaluate highly bioaccumulation substances since there needs to be more experimental data. Therefore, METI decided to use the dietary exposure test to identify non-highly bioaccumulative substances.

According to a provisional agreement, test methods were adopted that are similar to the OECD TG 305, but with three additional points. 1) Dietary exposure tests are applicable in chemicals which have water solubility bellow 0.01 mg/L and log $K_{OW}$ higher than five or for chemicals which are not stable in water. 2) Test diets have a lipid content of between 10% and 15%. 3) It is recommend to add reference substances to both control and treatment diets. As for bioaccumulation criteria in the provisional agreement, there is no definitive criteria if it is highly bioaccumulative. If it is not highly bioaccumulative, then growth-corrected kinetic dietary the $BMF_{Kg}$ should be less than 0.007. If $BMF_{Kg}$ is greater than or equal to 0.007 then it is left up to an expert judge to consider the accumulation to edible part and/or results of reference substance, etc.

In the field, $BMF_L$ equals the accumulated chemical in predator divided by accumulated chemical in prey. A $BMF_L$ greater than one indicates biomagnification, while a $BMF_L$ of less than one indicates no biomagnification. In the lab we can use a similar formula for the chemical concentration in fish where the source of the chemical is spiked feed. However, the feed content will be an artificial concentration, so you will end up with similar BMF but with a different meaning. Therefore, we can think of not using a BMF value of one as the standard. Therefore, at CSCL the BMF/BCF correlation is used to calculate the figures. Furthermore, for the provisional agreement on bioaccumulation criteria, the current criteria for not being highly bioaccumulative corresponds to a BCF of 1000, which is a $BMF_{Kg}$ of 0.017. Meanwhile, intra- and/or inter-individual variation of $BMF_{Kg}$ is approximately 2.4 times $BMF_{Kg}$, which means that it is not highly bioaccumulative if $BMF_{Kg}$ is less than 0.007.

In conclusion, if water solubility less than 0.01 mg/L and log $K_{OW}$ is greater than five then it is difficult to conduct the aqueous exposure test. In this case, the dietary exposure test is conducted by adding a reference substance to both control and treatment groups. If the concentration of the reference substance does not differ greatly between the control and treatment group fish and the $BMF_{Kg}$ is greater than 0.007 then determining whether the substance is bioaccumulative is left up to an expert judge. If $BMF_{Kg}$ is less than 0.007 then the substance is determined to be not highly bioaccumulative.

**Questions**

1. *When you conducted the BMF test you used fish oil, corn oil, and acetone but in the case of acetone the substance will only stay on the surface of the feed since it is volatile. Is adsorption a factor here?*

Only two tests used acetone. The lipid content is low, so that is why we think $BMF_{KgL}$ is low. Is bioavailability different between the tests? This is a conversation to have in the future, but it is possible.

2. *If you spike the feed then the test substance may be released into the water. What is the possibility of that and what will be different because of it?*

The release to water will be higher with spiked feed, but the substance is not water soluble. We measured the amount of substance in the water, but it did not reach the detection limit using the flowing water method, so accumulation of the substance in fish from water is minimal.

# Environmental Monitoring of cVMS in Japan

**Yuichi Horii**
*Center for Environmental Science in Saitama (CESS)*

Silicone is widely used in our lives and is used in construction, electric and electronic apparatuses, personal care and lifestyle items, transport equipment, and in general industry, so there is a need to understand the impact of cVMSs on health and the environment in the Japanese context where there is less information. cVMSs are very volatile, so 90% of emissions are into the air. Most of the remaining emissions are through the sewage treatment facilities. Although most cVMSs are eliminated, some may be accumulated in the environment. We investigated the distribution of atmospheric siloxanes. We placed focus on analyzing accumulation in aquatic environments and at sewage treatment plants we looked at the behavior of these substances as well as emissions into the air. In order to assess the accumulation of cVMSs we need a sophisticated approach and we have based our methodologies on ISO standards.

**Determination of VMS in the aquatic environment**

We used the purge and trap extraction method in determining VMSs in the aquatic environment. We targeted seven VMSs, the cyclic D3, D4, D5, and D6 as well as the linear L3, L4, and L5. Since siloxanes are present in much laboratory equipment, it was difficult to have high sensitive and high precision analysis without good blank control. We had to use silicone-free materials, analysts had to take great care in sample handling to reduce contamination, and carried out daily blank checks that include instrumental, procedural, and trip blanks. In addition, due to the high volatility and adsorption of VMSs into organic carbon, we applied PT extraction with ultrasonic assistance to get good extraction efficiency from aqueous and particulate phases. For purge and trap extraction, the sample liquid is put into a one-liter gas wash bottle. Then the sample is purged using a vacuum pump. The sample is therefore gasified and trapped in this absorber and then measured after elution with small volume of hexane.

**Investigation of sewage treatment plants (STPs)**

Through STPs some substances are emitted into the environment. We decided how to look at how siloxanes move in facilities and how much is eliminated. We sampled 25 STPs for D4, D5, and D6. The concentrations are shown in figure 1.

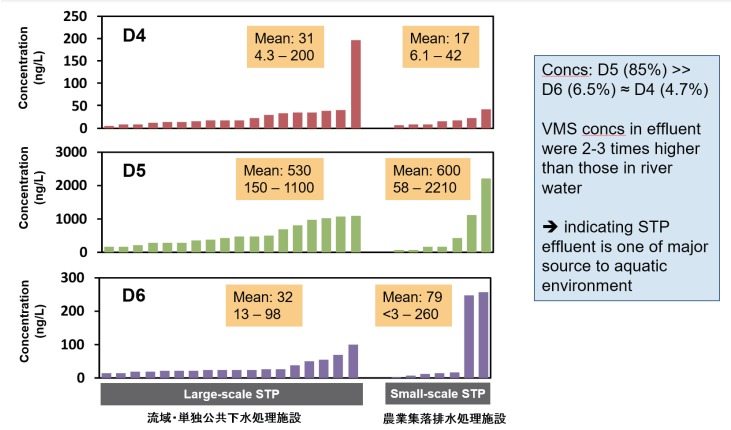



Figure 1: Concentrations of cVMS in effluent from 25 SPTs

Small-scale STPs indicate rural plants serving around 1000 people. D5 accounted for 85% of the total with 530 ng/L being the mean concentration, which seems to be twice or thrice the values found in the river environments, so STPs seem to be the main source of emissions. Next we looked at the Mass balance of D5 in a sewage treatment plant as shown in figure 2.

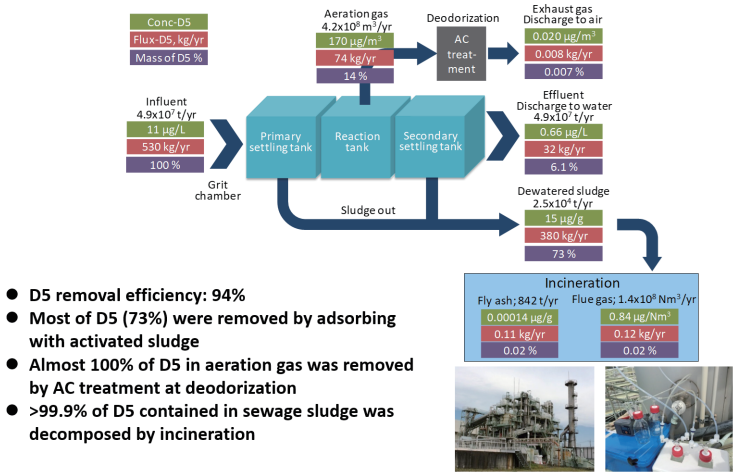



Figure 2: Mass balance of D5 in a sewage treatment plant

D5 removal efficiency was 94% with most of D5 (73%) being removed by adsorbing with activated sludge. Almost 100% of D5 in aeration gas was removed by AC treatment at deodorization, and more than 99.9% of D5 contained in sewage sludge was decomposed by incineration. We estimated the emission of D5 via STP effluent around Tokyo Bay and found that there was a total emission of about 2000 kg per year. The Sumida River having the highest share at 380 kg per year and the Arakawa River at 320 kg per year. Numerous STPs around Tokyo Bay emit water into Tokyo Bay for a total of about 800 kg per year of emissions.

## VMS monitoring in Tokyo Bay watershed

We took 84 samples from river that had an average total concentration of 212 ng/L for the seven VMSs we tested for. In Tokyo Bay the concentrations were very low at 11 ng/L,

which is close to the measurable limit. In downstream STPs in the urban area high concentrations were observed. In the northern part of Saitama Prefecture septic tanks are still used and concentrations were very high. In the downstream of rivers, near the river mouths concentrations of siloxanes in sediment were found to be high. Therefore, high accumulation of siloxanes were observed in the sediment.

Figure 3 shows the relationship between cVMS concentrations (ng/L) and suspended solids (SS) (mg/L) or total organic carbon (TOC) (mg/L).

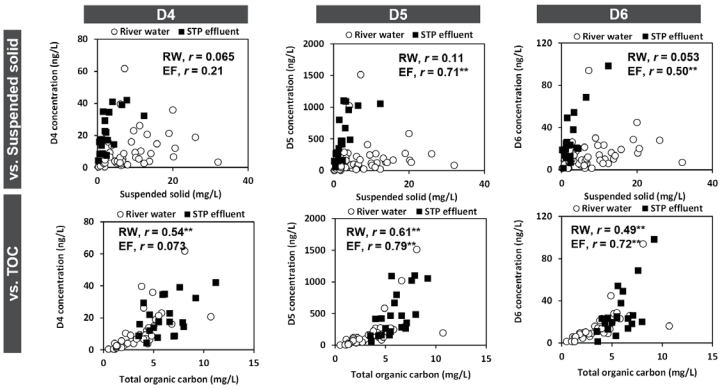



Figure 3: Relationship between cVMS concs (ng/L) and suspended solid (mg/L) or TOC (mg/L)

There was no correlation with SS in river solids, but significant correlation with SS in effluent, as well as significant correlation with TOC in river water and effluent, which is dependent upon the amount of organic materials found in effluent. We have published a paper on this topic in the journal *Science of the Total Environment* titled 'Distribution characteristics of volatile methylsiloxanes in Tokyo Bay watershed in Japan: Analysis of surface waters by purge and trap method'. If you are interested, please read the paper.

**Fish investigation in Tokyo Bay and inflow rivers**

In rivers we tested carp and black bass. In Tokyo Bay we looked at sea bass and eel as high trophic level samples. As for concentration profiles of VMS (μg/g-lipid) in fish samples, the highest values were found near the discharge point of STP water. Therefore, the location is an influencing factor with concentrations lower in Tokyo Bay as compared with rivers. We did not find high concentrations in high trophic level samples.

**Regional characteristics and temporal trends of methylsiloxanes in the atmospheric environment, Saitama, Japan: simultaneous analysis for 20 compounds**

We determined cyclic methylsiloxanes D3 through D9 and linear methylsiloxanes of L3 through L15 in the atmosphere. We used a small mass-flow pump attached to an SPE cartridge to capture siloxanes at nine locations in Saitama Prefecture over one week. We have conducted continuous measurements since June, 2016. We divided the prefecture into the three sections of urban, mountainous, and agricultural. We find higher concentrations of methylsiloxanes in the south of the prefecture and lower concentrations in the north with D3, D4, and D5 accounting

for the vast majority of emissions and D5 having the highest concentrations especially in urban areas due to its use in aerosol products. However, D4 concentrations were higher in the northern agricultural region, which may be due to differences in half-life between D4 and D5, or there may be a source of D3 and D4 that we are not yet aware of.

We used the AIST-ADMER model to estimate D5 emissions from the use of personal care products that 281 grams are emitted per day. High levels of emissions were found in the urban area, southern Saitama Prefecture. The concentration distribution and the emissions scenario of D5 seems to match.

At CESS we do continuous monitoring throughout the year. We found concentrations in the range of 100 to 960 ng/m$^3$ and an average of 390 ng/m$^3$. We have seen that concentrations vary over the year with the highest numbers being found in autumn and winter and the lowest in summer. Interestingly, the ratio of D4 and D5 is low when concentrations are low and high when concentrations are high.

Finally, figure 4 shows the compositions of cVMSs in each environmental media.

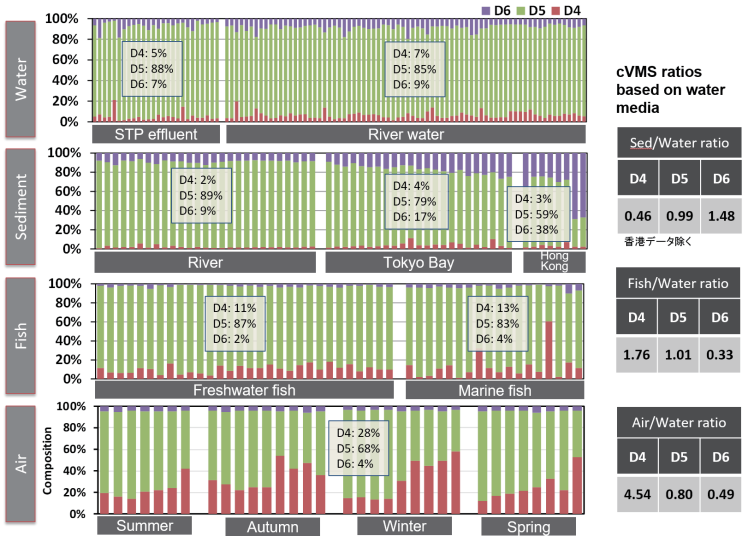



Figure 4: Compositions of cVMS in each environmental media

In each medium D5 has the highest percentage since it is added to personal care products. Interestingly, however, those proportions were slightly altered in each environmental media, depending on the physico-chemical properties of cVMS.

**Further questions**

What are the temporal trend for cyclic and linear? Will they increase, decrease, or not change? What is the composition of cVMS in dissolved and particulate phases in a water environment? This is an important factor to predict environmental fate and bioavailability of cVMS. What is the factor for seasonal variations of cVMS (D4) in the atmosphere? What are the D3 sources in the environment? Is it from impurities in silicone products or any specific source, or do they originate from degradation or transformation in the atmosphere?

**Questions**

1. *In water and fish you investigated the concentration and you have looked at the bioaccumulation factors. Have you looked at the ratio between fish and water?*

This looks at the changes in ratio between fish and water. For BAF, the ratios ranged several thousands to tens of thousands different in concentrations seen.

# Cyclic Volatile Methyl Siloxanes: Environment Canada D5-Board of Review and the Science and Process Used in Risk-Based Weight of Evidence Assessment of cVMSs

**Keith Solomon**
*Centre for Toxicology, University of Guelph, Canada*

As background, it is important to keep in mind that all things are toxic and can present a hazard, but that no or minimal exposure is equal to no harm. It is when exposure above a certain amount is reached that there will be harm. It is when you include probability into the mix is when you will be able to characterize risk (Figure 1).

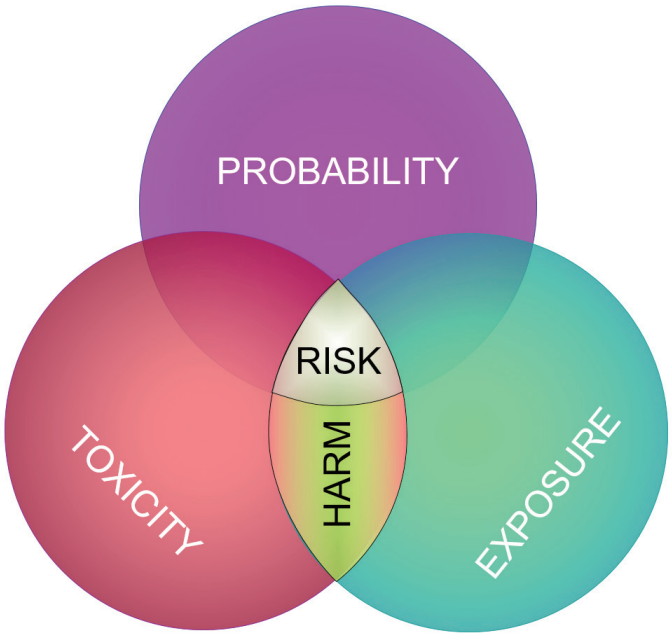



Figure 1: Probability must be considered to characterize risk

Risk is totally ignored in current POPs and PBT regulations. It must have a component of probability of exposure and sensitivity. The only way that risk can be managed is to reduce intensity and probability of exposure.

## Legislative background

The legislative background of the D5 board of review (BoR) is the Canadian Environmental Protection Act (CEPA) of 1988, and the Toxic Substances Management Policy 1995. These were adopted to protect the environment and human health by the virtual elimination of substances that are toxic, persistent, and bioaccumulative, as well life-cycle management of

other toxic substances and substances of concern to prevent or minimize their release into the environment. The Canadian Environmental Protection Act of 1999 detailed screening assessments of substances to determine if they present risk to the environment or human health with three outcomes: no further action, further assessment, or virtual elimination.

**Siloxane D5 board of review**

CEPA Screening concluded in November 2008. D5 was entering the environment in a quantity concentration or under conditions that may have harmful effect on the environment or its biological diversity, so it was to be added to schedule 1 of CEPA. In July 2009, an affected industry stakeholder filed a Notice of Objection and requested that a BoR be established. In August 2010, the Minister of the Environment published notice of his intention to establish a BoR to review D5 but not D4. The members of the BoR were John Giesy, Keith Solomon, and Sam Kacew. The key question for the board was whether D5 presented a 'danger to the environment'. Based on legal precedent, this was determined to require "…a de novo assessment of risks posed by D5, taking into account all of the available, relevant, scientific information…" The members of the board had judicial status. Witnesses were called by counsel for Environment Canada, the appellants, and the BoR. Witnesses were cross-examined by counsel for EC, the appellants, and the BoR. The BoR then produced a report that was provided to the Minister of the Environment.

The BoR on D5 concluded that D5 exceeded the regulatory threshold for persistence, did not exceed the thresholds for bioaccumulation, does not biomagnify through the food chain, that there is no evidence to demonstrate that D5 is toxic to any organisms tested up to the limit of solubility in any environmental matrix, that D5 does not pose a danger to the environment, and that projected future uses will not pose a danger to the environment. Aside from the legal aspects of the BoR, the panel followed good scientific methods for review and analysis, used probabilistic risk assessment for their assessment, recommended the use of transparent modelling approaches, and did not use weight of evidence in the formal sense.

**Weight of evidence (WoE)**

The term weight of evidence originated in the legal system, but as a process has been misused in the scientific arena. WoE is widely used but seldom formalized. WoE has three characteristic uses in the literature related to risk assessment for human health. The first is metaphorical where WoE refers to a collection of studies or to an unspecified methodological approach. Most uses of WoE have been metaphorical and no better than a magician's wand. The other two characteristic uses are methodological where WoE points to established interpretative methodologies, and theoretical where WoE serves as a label for a conceptual framework.

As scientists, we are faced by the data paradox. Published studies are often incompletely documented, raw data is rarely available, most studies do not follow standardized protocols, there is selection bias, and there is publication bias since negative results are not published. Therefore, studies need to use good laboratory practice, have quality assurance, and quality control. This is required by regulation and means that studies are completely documented,

that raw data is available, standardized protocols are used for most studies, and that there is no selection or publication bias.

**Quantitative WoE (QWoE)**

There is too much data with a large number of studies reported in the literature, some showing adverse effects and some not. Proponents for each side of the question will select conclusions from studies that support their position. This results in arcane arguments from experts involved in a 'he-says—she-says' debates. QWoE is the exit strategy. However, QWoE is not about criteria. Criteria for protection of the environment use data on P, B, T, etc. The best data are usually selected based on the quality (reliability) of the studies. Therefore, some studies are excluded. QWoE includes all studies that have enough data to allow quality to be assessed.

QWoE is not a simple scientific review, a meta-analysis, or risk assessment. Instead, QWoE assesses the consistency and coherence of data on the biological or environmental behavior of a stressor (chemical or otherwise). QWoE can address risk but not yet in the fully probabilistic sense. Observed responses that are relevant can be compared to criteria, NOECs, or probabilistic values. QWoE does provide a consistent and transparent process to bring all relevant studies together with minimal bias and to show overall relevance. The process of QWoE assessment is shown in Figure 2.

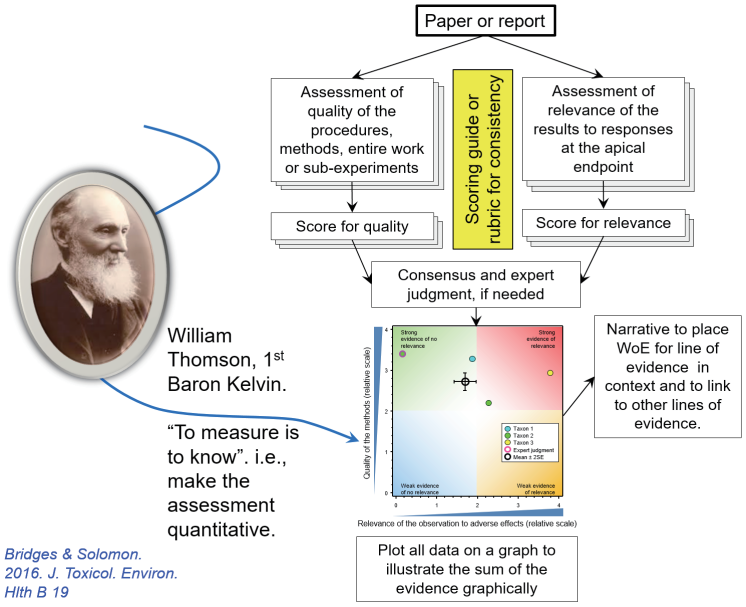



Figure 2: Process of QWoE assessment

As for scoring for quality, the scoring guide acts as a filter based on the methods and reporting of a study to assign a quality score. Studies with a high score are good studies for setting criteria, while those with a low score are unsuitable for setting criteria. However, regardless of the score, all studies are used for QWoE. QWoE is about maximizing consistency and transparency so the reasons for the scores are provided. There are two components of a QWoE, the narrative and the supplemental information (scoring sheets).

The results of QWoE assessments for TMF data for D5 and toxicity data for both D4 and D5 are shown in Figures 3 through 5.

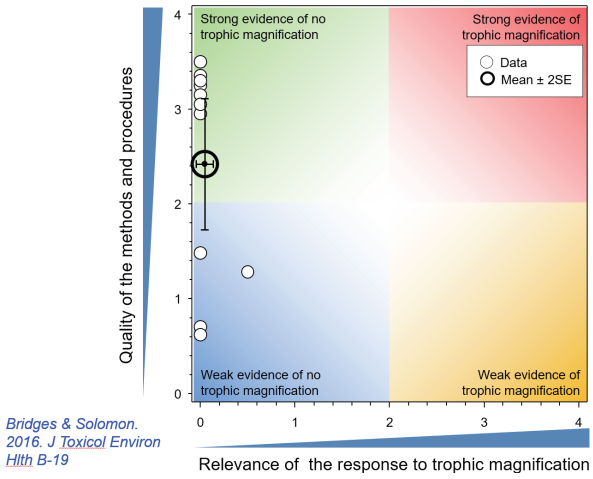



Figure 3: Example of TMF data for D5 ($n_R$=11)

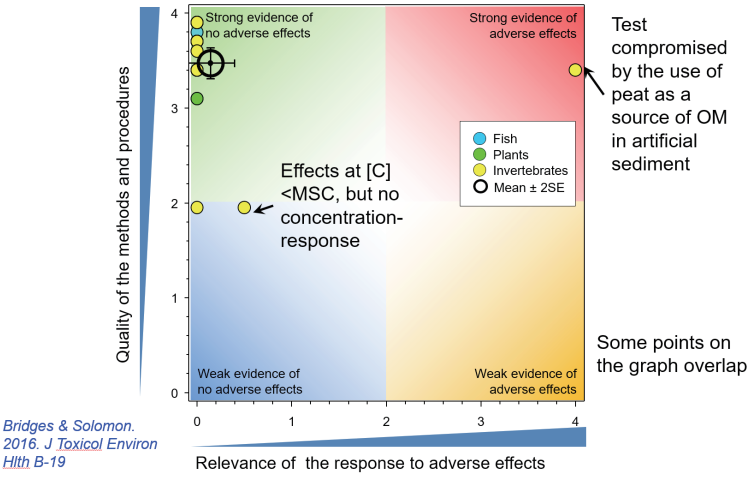



Figure 4: Example of toxicity data for D4 ($n_R$=32)

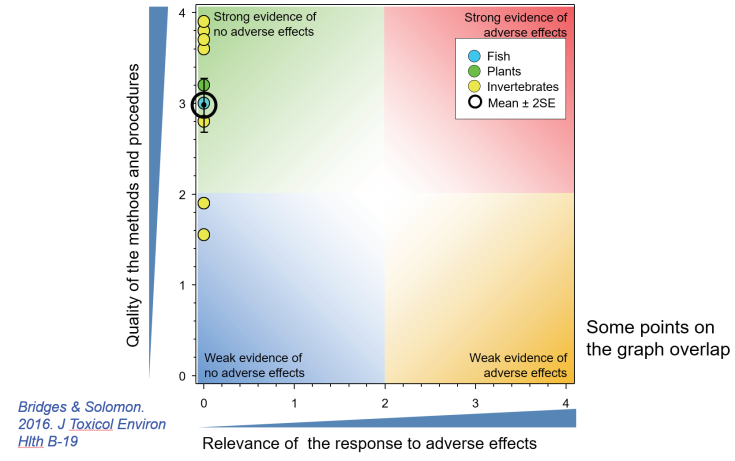



Figure 5: Example of toxicity data for D5 ($n_R$=36)

Conclusions of the QWoE are as follows: 1) cVMSs are persistent in some studies in some matrices (sediment) but it is $P_{overall}$ or P in the final compartment (air) that matters. 2) Studies in food-webs support a conclusion that the cVMSs do not meet the criteria for biomagnification; consistent with toxicokinetic studies and modelling. 3) Toxicity of cVMSs was not observed less than or equal to solubility in water or the maximum sorption capacity in soils and sediments. Concentrations in the environment were not measured at greater than or equal to the solubility in water or the MSC. 4) Concentrations in air in remote locations are small, and if deposition occurs it is very unlikely to be biologically relevant.

# Ecological Risk Assessment of cVMS

**Ellen Mihaich**
*Duke University and Environmental and Regulatory Resources, LLC*

## Risk assessment methodology

Risk is a function of both hazard and exposure. Risk assessment is the use of the factual data base to define the health or environmental effects of exposure of individuals or populations to materials or situations. There are four steps involved. The first is problem formulation or hazard identification to find out what is known about the situation or material and to develop hypotheses. The second is the characterization of ecological effects or a dose-response assessment to determine the inherent toxicity under defined conditions. The third is an exposure assessment to determine how much is out there. The final step is risk characterization to determine how risky something actually is by comparing the toxicity and exposure components.

## Overview of risk assessment process for cVMS

The chemical properties of cVMSs are shown in figure 1.

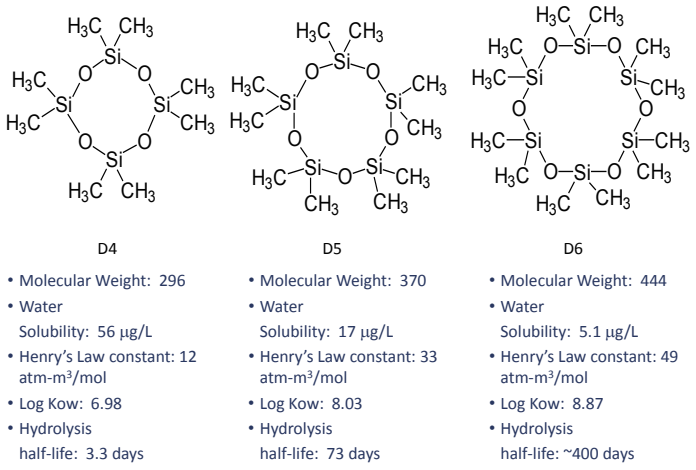



Figure 1: Chemical properties of cVMSs

As background, cVMSs are a very well-researched class of materials. Through their manufacturing, handling, use and disposal, traces of cVMS may enter sewage treatment plants and receiving waters. Due to their properties, accumulation in sediments is possible and volatilization is likely. Assessing ecotoxicity, exposure, and risk in the water and sediment compartments is warranted but challenging. Problem formation is based on protection goals to

protect human health and the environment from adverse effects and involves the development of working hypotheses as to how cVMS might distribute in the environment and affect components of the ecosystem.

The properties of D4, D5, and D6 exert control over test designs. First, they have low aqueous solubility so it is a challenge to prepare test solutions and a challenge to conduct analytical measurements. They have a combination of low water solubility, high $K_{OW}$, and high Henry's Law values, and therefore have a tendency to escape the water through sorption and volatilization but not redeposit once in the air. In order to conduct aquatic tests, closed, no-head space, flow-through test systems (except for algae) are generally used. In addition, it is important to make careful measurement of test concentrations, understand the maximum sorption capacity in the matrix, and consider water solubility.

cVMSs have unusual properties that constrain exposure. Despite unusually small $K_{OC}$, they partition to organic carbon in soil and sediment but not at concentrations greater than the maximum sorption capacity. This limits exposure in toxicity tests. Data from tests conducted at concentrations above the maximum solubility or MSC are not ecologically relevant.

**Results of cVMS toxicity testing**

To summarize aquatic toxicity results, for D4 there were no acute 96-hr effects at solubility limits for fish, invertebrates, and algae. The lowest 14-day LC50 for rainbow trout is 0.010 mg/L in an enclosed system. There was a marginal effect on Daphnia survival at the highest test concentration in a 21-day study with no effect on reproduction. For D5 there were no effects at solubility limits in acute and chronic tests with fish, invertebrates, and algae, and no effect on fish in the dietary dosing study. For D6 there were no effects at solubility limits in acute and chronic tests with fish and invertebrates.

To summarize sediment toxicity results, for D4 there were NOECs of 13 to 44 mg/kg dw in chronic tests in natural sediments with synchronized *Lumbriculus* and *Chironomus*. There was also no obvious effect of varying OC in 14-day tests with *Chironomus* with a NOEC of 54 to 120 mg/kg dw and an LC50 of 130-170 mg/kg dw. For D5, acute and chronic tests with *Hyalella* and *Chironomus* gave similar results of 69 to 130 mg/kg dw based on survival or development. *Lumbriculus* was relatively insensitive to D5 with NOEC equaling 1272 mg/kg dw. For D6 there were no effects on *Lumbriculus* at the highest concentration tested of 484 mg/kg dw, and no effects on other benthic species are expected.

The D5 avian reproduction study followed OECD 206 guideline with Japanese quail. There were no statistically significant effects observed on any parameter measured during the 22-week JQ reproduction study with D5. The NOEC during the study was 1000 mg/kg feed, or 143.5 mg/kg body weight per day. The NOEC determined in the D5 avian reproduction study is at a concentration approximately 10,000 times higher than what has been measured in soil, sediment, and fish samples in the environment.

**Exposure assessment**

Models are being developed to consider unique properties of cVMSs, and monitoring data will help validate models. Field monitoring is ongoing around the world, for example for the Voluntary Product Stewardship Initiative in Inner Oslo Fjord, Lake Ontario, Lake Pepin, and Tokyo Bay. Challenges in cVMS trace analysis include contamination issues during sampling, transport, storage, and analysis. Quality control measures are also critical since the measured data are generally at or below detection limits.

The critical body burden (CBB) is the lowest total body concentration of a chemical which is associated with adverse toxic effects. The first order pharmacokinetic model is: $C_{fish} = K_1/k_2 * C_w * (1\text{-}exp\text{-}(k_2+k_m) * time$. As for predicting chronic NOEC using CBB, rainbow trout NOEC from the early life-stage study is 4.4 µg/L at the highest concentration tested and the high concentration was based on the acute study. Using the CBB model, the exposure time to reach a concentration that would cause adverse effects ranges from 27 to 269 days depending on fish size and concentration. For the 93-day trout ELS, concentrations equal to or less than 12 µg/L could have been used without adverse effects occurring. Mortality after seven days in the acute study was unexpected and may indicate issues with the non-standard test conditions.

As for pelagic risk characterization of cVMSs, the compounds exhibit low toxicity to pelagic species. Field $C_{biota}$ is much less than CBBs, and NOECs are often greater than water solubility as shown in figure 2.

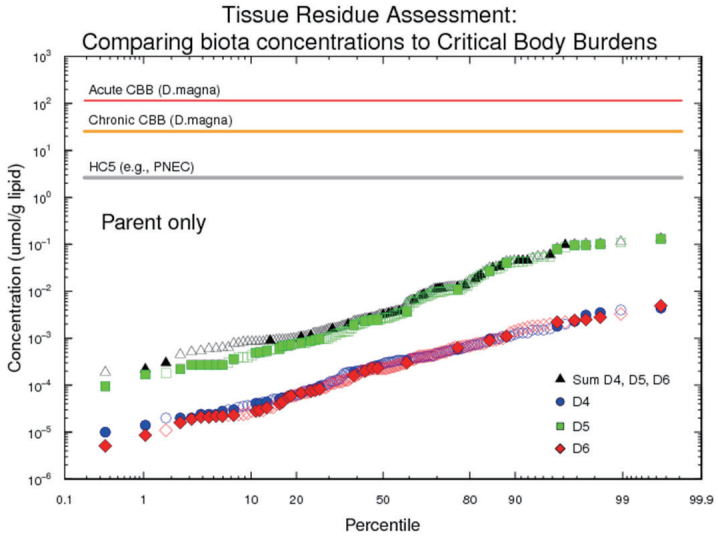



Figure 2: Pelagic Risk Characterization of cVMS

As for sediment risk characterization with D4, figure 3 shows Probability Density Function (PDF) and Cumulative Distribution Function (CDF) for D4 field sediment exposure and chronic benthic invertebrate toxicity NOEC values (mg/kg-OC) in a benthic species sensitivity distribution.

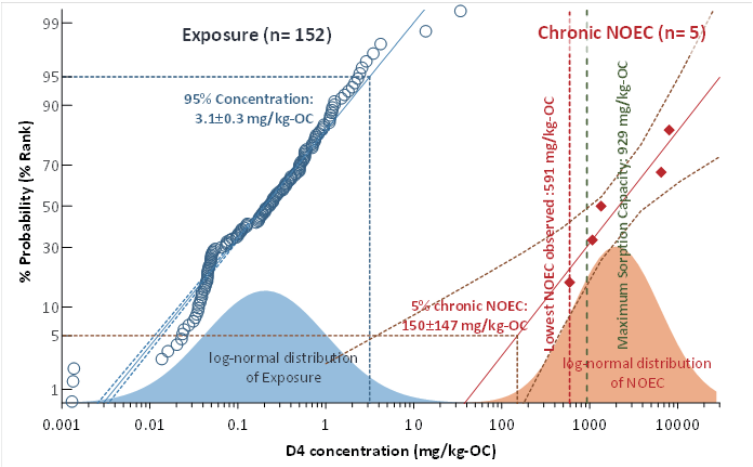



Figure 3: Sediment Risk Characterization with D4

As for sediment risk characterization with D5, figure 4 shows PDF and CDF for D5 field sediment exposure and chronic benthic invertebrate toxicity NOEC values (mg/kg-OC) in a benthic species sensitivity distribution.

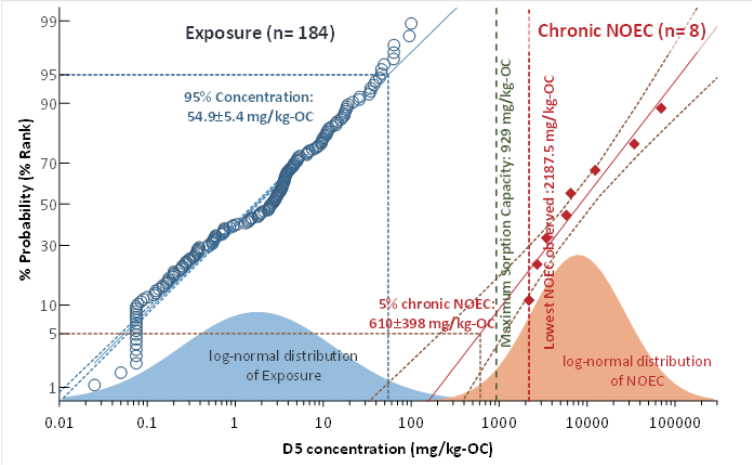



Figure 4: Sediment Risk Characterization with D5

The fact that exposure and chronic NOEC in the previous two graphs do not overlap indicates low risk. Results were also the same for D6 at low concentrations.

## Conclusion

cVMSs have robust ecotoxicity and monitoring databases and have unique physical and chemical parameters that limit the chance for significant exposure in the environment.

Concentrations in the environment are low and cVMSs preferentially partition to sediment and air. Probabilistic effects and exposure assessments do not indicate the risk to aquatic or sediment organisms. Finally, using a quantitative WoE procedure and risk assessment, it can be concluded that cVMSs do not present a risk to human health or the environment when used as intended.

**Questions**

1. *I understand that siloxanes are safe with no risk, but there is one study that is an outlier. Could you explain why you reject this data?*

The problem with the *Lumbriculus variegatus* study was that the organic material for the artificial sediment was not the best when the test was being made up. Also, the worms were not synchronized by cutting off their heads, which was at odds with the guidelines. Therefore, there is a lot of variability that could have caused this result by chance. That is why we did another study with natural sediment and made sure that the worms were synchronized per the guidelines.

2. *Could you explain about the relationship between the plots of quality score and the graph in figure 4?*

We use all of the monitoring data we have to develop a cumulative distribution or a probability density function. The quantitative weight of evidence helps us know how everything fits. We developed a regression and then plotted the lowest no effect level. This is probabilistic since you have distributions with the 95$^{th}$ percentile of concentration and the 5$^{th}$ percentile of the effect, so this is very conservative.

# Pharmacokinetic Behavior of D4 and D5 in Fish and Mammals: Why D4 and D5 Do Not Accumulate

**Kathleen Plotzke**
*ICCA-LRI Chair, Dow Chemical USA*

### The concern with bioaccumulation: biomagnification

It is important to determine whether a chemical movement in food webs magnifys or biodilutes. For high uptake or a non-metabolizing chemical there will be an increase in concentration as it moves up in trophic levels, while low uptake rate or highly metabolized chemicals will decrease in concentration as it moves up the trophic levels. The reasons why a standard BCF is not a good measure for biomagnification of cVMS is because standard laboratory BCF tests fail to accurately predict the ability of a highly lipophilic, poorly water soluble, and metabolizing substance such as D4, D5, and D6 to biomagnify or not in the environment. Dietary exposure is the main route of uptake for bioaccumulation of highly lipophilic substances in the environment. Understanding uptake, metabolism, and elimination of these substances in different organisms in the environment is critical for understanding biomagnification potential.

### Processes affecting biomagnification in aquatic organisms

Mackay et al. in 2015 examined the importance of the role of physical-chemical properties in evaluating the potential for accumulation from 'superhydrophobic' compounds such as cVMSs. The term 'superhydrophobic' is applicable to compounds with log $K_{OW}$ greater than about seven, which includes D4, D5, and D6. The authors compiled a series of conventional uptake equations and a simple accumulation model for aquatic organisms. Among the important factors that were examined were the roles of hydrophobicity and metabolism. Bioaccumulation results when uptake is significantly greater than elimination. As for the processes affecting biomagnification in aquatic organisms, ADME processes are critical. Biota concentration of a chemical equals uptake through the gills and diet minus loss via the gills, egestion, and metabolism.

Taking the example of D4 metabolism in fish, metabolism in vivo has been measured in lab studies with rainbow trout as shown in figure 1. Very little to no parent D4 is found in bile or urine respectively following administration of D4 to rainbow trout. This example of a 96-hour trout metabolism study following bolus doses also had estimated $k_M$ of greater than 0.01 per day..

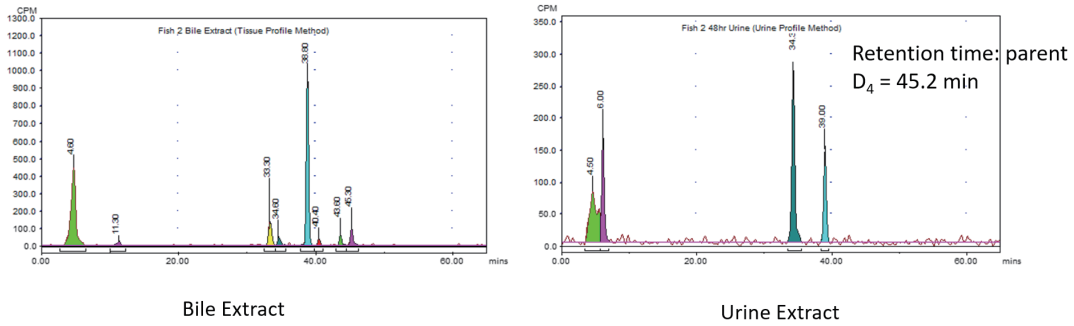



Figure 1: D4: Metabolism in Fish (example)

Results of this study were similar with D5. Clearance occurs via enterohepatic circulation of metabolic products in bile with excretion via the digestive tract and urinary clearance of metabolites. D4 and D5 are metabolized in rainbow trout with similar estimated biotransformation half-lives of less than seven days. There is biodilution of cVMS in food webs due to metabolism and/or lack of uptake. Results on cVMSs both in vitro and in vivo demonstrated metabolism in fish. The silicone industry is funding additional metabolism work performed via Henriette Selck of Roskilde University on benthic organisms and Frank Gobas of Simon Frazer University on fish regarding more environmental relevant dosing and concentrations.

**Processes affecting biomagnification in terrestrial organisms and humans**

Again, ADME processes are critical for understanding biomagnification potential in terrestrial organisms and humans. Biota concentration equals uptake via skin/inhalation plus diet minus loss via exhaled air, metabolism, and egestion. This is a simple mass balance process where bioaccumulation results when uptake is significantly greater than elimination. Extensive pharmacokinetic studies in mammals for D4 and D5 (including rodents and humans) demonstrate that D4 and D5 are readily eliminated either by exhalation in breath or via metabolism to polar metabolites excreted in urine. It is not possible for D4 or D5 to bioaccumulate due efficient elimination processes in mammals. This process is detailed in figure 2.

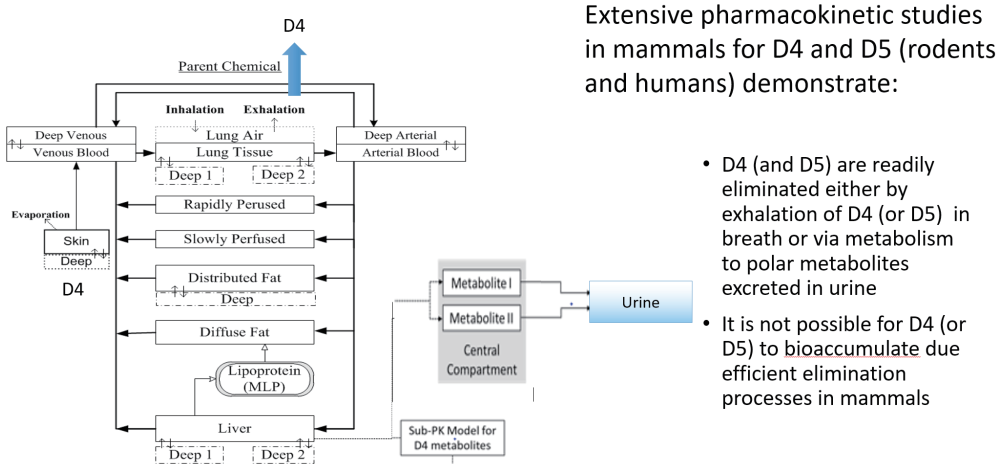



Figure 2: D4: Exhalation and Metabolism in Mammals (example)

Figure 3 shows the mammalian urinary metabolite profile post exposure to D4 and shows that no parent D4 is found in urine. Similar results were seen following exposure to D5.

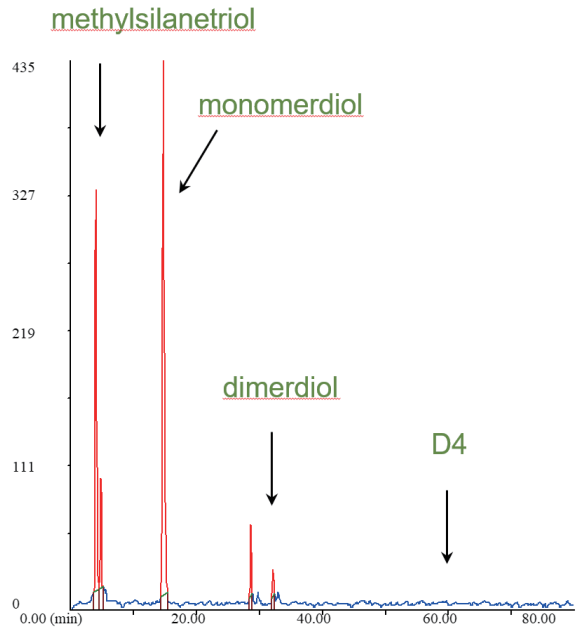



Figure 3: Mammalian Urinary Metabolite Profile Post Exposure to D4

**Summary**

cVMSs undergo metabolism/biotransformation in a salmonid species, the rainbow trout. Further studies are ongoing with fish and benthic invertebrates under relevant exposure/dose conditions in the environment. cVMSs are readily metabolized or exhaled in terrestrial organisms, and field data indicate biodilution. cVMSs are readily metabolized or exhaled in humans and concentrations will not increase over time.

**Questions**

1. *For the fish metabolism study, how did you calculate the bio-transformation half-lives? Are these values from the whole body?*

We looked at the whole body as well as the blood concentrations over time. It is similar to what we do for mammals.

2. *I think you suggest low bioaccumulation of D4 or D5 by fish, but this morning we were told that the bioaccumulation factor is around 5000. What do you think about this point?*

With the BCF, you are maintaining concentrations of these substances in the water, you will get partitioning of these lipophilic substances into lipophilic fish. However, in the environment the concentrations in the water re low so this will not be a major exposure pathway. However, you do find these substances in the sediment, therefore, it is important to look at exposure through the diet where metabolism will play a significant role

3. *Can we think that this is different from traditional POP substances?*

It is very different since metabolism plays a key role for these substances where the traditional POP substances were not readily metabolized

4. *What kind of reaction is happening in the metabolism of these substances?*

It is enzymatic metabolism. We have studied this in detail in the rodents. It is a P450 enzyme demethylation, which destabilizes the ring. Then it undergoes simple hydrolysis.

5. *When D4, D5, D6 are metabolized you have alcohol which is soluble in water. What is the toxicity of these compounds? You may need to think about the long transport characteristics of these substances as well.*

From a toxicity perspective, we have done acute aquatic toxicity tests and you see nothing even at the highest concentrations tested. From long-range transport or PBT perspective, they are highly water soluble and have very low log $K_{OW}$ so they will not bioaccumulate. . They might be found in the atmosphere in remote regions but that would be from degradation of cVMS. .

6. *CYP P450 is an enzyme in the liver, and for just-hatched fish CYP levels are low, so I am concerned for juvenile fish. Do you have evidence of low toxicity in juvenile fish?*

The real key is the exposure component. You will not have enough exposure for it to build up to achieve a narcotic mode of action, which is the only mode of action that would cause adverse effects. You have to get to high concentrations to achieve that.

# Hazard Assessment and Health Risk Characterization for D4 and D5

**Wolfgang Dekant**
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### Available studies on the toxicity of D4 and D5

Guideline-compliant studies cover all endpoints considered relevant for hazard assessment. Studies were performed using inhalation exposure due to specific characteristics of absorption of D4 and D5 from the gastrointestinal tract after oral administration with micro-emulsions. Concentrations used in inhalation studies are limited by aerosol formation above approximately 160 ppm for D5 and approximately 700 ppm for D4. From the available studies, there were a number of endpoints affected in repeated-dose studies with D5. There were reversible increases in liver weight with a NOEC greater than 28 ppm in a 28-day study. There were increased lung weights and alveolar macrophage accumulation with a NOEC of 10 ppm in a 28-day study. There were no effects on development and fertility in one- and two-generation studies. There were small increases in the incidences of uterine endometrial carcinoma after two-year inhalation exposure at 160 ppm.

### Risk characterization for D5

Reversible liver changes due to the induction of CYP450 mediated by CAR are not considered as adverse, but represent an adaptive response due to absence of histopathological changes in liver. Mild lung effects represent the typical response to repeated inhalation exposures to a mild irritant. Induction of uterine tumors is caused by a dopamine-like mode of action altering the estrous cycle in the rat that increases exposure of the target tissue to endogenous estrogen.

As for risk characterization for lung effects, SCCS used the NOAEC of 49 ppm for local effects on the respiratory tract as a point of departure (POD) for risk characterization after inhalation exposures to D5. SCCS compares air concentrations of D5 using a POD of 740 mg/m$^3$ to derive the margin of exposure/safety. The margin of safety/exposure (MoS, MoE) calculation needs to be based on a comparison of the dose of D5 received per unit of lung weight in the inhalation studies and dose of D5 received per unit of lung weight in consumers. The local effect is determined by dose to target. In addition, the particle sizes of D5 aerosols needs to be considered since only small particles penetrate into the lung.

Local respiratory tract PoD was derived from a 90-day vapor inhalation study with D5. PoD equal to a NOAEC for lung effects is 49 ppm or 0.75 mg/L. Inhalation volume in male rats is 20.5 L/h. Exposure time is six hours per day and was converted from five to seven days per week. The average lung weight in male rats in the study was 1.02 g. The resulting inhalation

PoD was 65 mg of D5 per gram of rat lung per day.

The calculated consumer respiratory tract exposure to D5 from inhalation of aerosols products and margins of exposure to adjusted PoD in rats shows that there is no risk from D5 from hair styling aerosols, hair treatment aerosols, and deodorant sprays.

As for uterine tumor induction, D5 is not genotoxic, not estrogenic, and does not induce a cytotoxic response in the uterus. The dopamine-like activity of D5 results in increased number of estrogenic days in aging Fischer 344 rats. However, tests did show increased estrogen exposure as shown in figure 1.

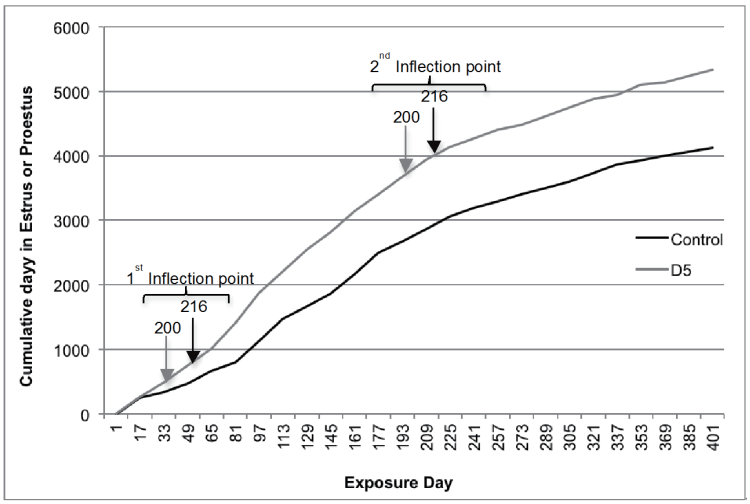


Figure 1: Uterine tumor induction

**Endpoints affected in repeated-dose inhalation studies with D4**

The hazard data was very good. There were reversible increases in liver weight similar to D5. There was also slight nasal mucosal goblet cell proliferation and interstitial inflammation of the lung and increased kidney weights. Although there was chronic progressive nephropathy, this does not happen in humans and is not relevant. There were no effects on development, but in Sprague Dawley rats there were decreased numbers of implantation sites and a reduced mean live litter size at 700 ppm in a one-generation reproductive toxicity study by whole body inhalation of D4 for six hours a day a minimum of 28 days prior to mating and through the day. Exposures of females suspended from GD 21 to lactational day four and offspring were examined after sacrifice on PND 28.

There was also a two-generation reproductive toxicity study by whole body inhalation of D4 for six hours a day for at least 70 consecutive days prior to mating through weaning of pups on postnatal day 21. This study showed reduced mating and fertility index in F1 at 700 ppm, a reduction in mean live litter size and mean number of pups in F0 and F1 at 500 ppm and 700 ppm, an increased estrous cycle length in F1 females at 700 ppm, and a reduction in corpora lutea and reduced numbers of pregnancies at 700 ppm. There were, however, no adverse effects on male reproductive endpoints.

As for the effect of inhalation exposure to D4 for 24 months on uterine histopathology in Fischer 344 rats, the only statistically significant difference from control was an increase of endometrial epithelial hyperplasia at 700 ppm. A statistically significant trend for endometrial adenoma was also observed.

Figure 2 shows how the dopamine-like activity of D4 increases estrogen exposure of the uterus.

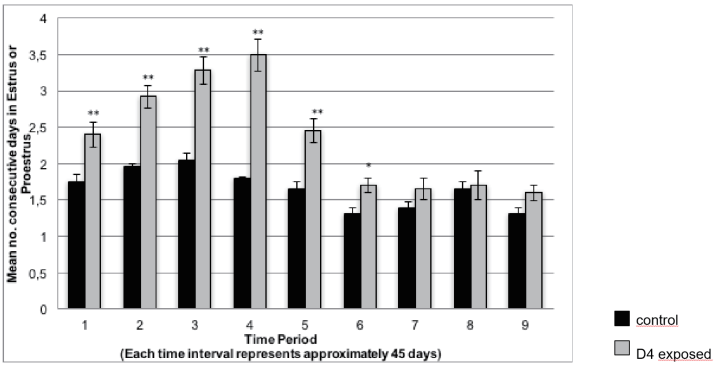


Figure 2: Dopamine-like activity of D4 increases estrogen exposure of the uterus

D4 was found not to be genotoxic, has only a weak affinity to estrogen receptors, and does not act as estrogen.

**Human relevance of uterine tumor induction by D4 by alteration of prolactin through a dopamine-like activity of D4**

Figure 3 shows the mode of action in rat from a decrease in prolactin that leads to endometrial hyperproliferation.

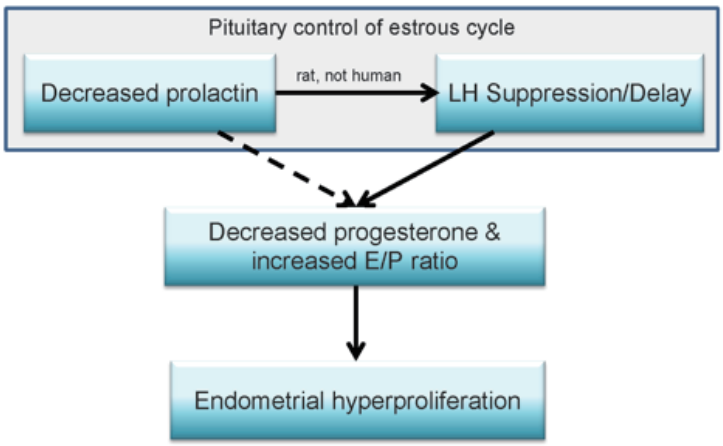


Figure 3: Human relevance of uterine tumor induction by D4 by alteration of prolactin through a dopamine-like activity of D4.

It is important to note, however, that the LH suppression/delay found in rat is of little relevance to humans. Indeed, figure 4 shows how the chain of events that leads to decreased mating and fertility in rats is broken at the point of decreased LH surge, since a decrease in prolactin is not associated with a decrease in LH surge in humans.

| Key steps in mode of action | Data support | Possible in humans |
|---|---|---|
| **Increased dopamine activity** | There are no data on D4 and dopamine in humans, but it is theoretically possible that a chemical exposure could increase dopamine activity, as do some pharmaceutical products. | 1 |
| **Decreased prolactin** | An increase in dopaminergic activity will decrease prolactin in humans | 1 |
| **Decreased LH surge** | A decrease in prolactin is not associated with a decrease in LH surge in humans. | 0 |
| **Inhibition/delay of ovulation and/or inadequate corpus luteum** | A sufficient decrease in LH surge will inhibit or delay ovulation in humans. However, control of LH is very different in rodents compared to primates and humans. | 1 |
| **Decreased mating, fertility** | Inhibition of ovulation will decrease fertility in humans. | 1 |
| **Total** | | 0 |

0, not relevant since specific step in mode of action is not possible in humans due to species differences in biochemistry/physiology/anatomy;
1, step possible based on human biochemistry/physiology/anatomy. If an early step in a mode of action is scored as 0, further downstream steps are not possible and the mode of action is therefore not relevant in humans

Figure 4: Human relevance of a dopamine-like mode of action of D4 regarding female fertility

## Summary

Modes of action have been developed for all toxicity endpoints for D4 and D5 relevant to risk characterization. Experimentally-supported modes of action for uterine tumor induction by D4 and D5 and for female-specific effects on fertility (D4) have no human relevance. Local effects of D4 and D5 on the lung may be used for characterization of health risks after inhalation using appropriate dosimetry.

## Questions

*1. If you were responsible for CLP classifications on these two materials, what would be your conclusion on the GHS classification?*

I would issue no classification. In Europe they would probably end up with a CMR 2 classification because you see tumors. Due to regulatory issues it is difficult to not issue a classification even in the absence of human relevance.

## Round Table/Wrap Up

**Questions:**

1. *Regarding cyclics in the environment and their degradation, could you talk about the mechanism and how stable cyclics are in the environment?*

2. *Regarding health, what are the respiratory effects on the body either in the nose or in the lungs?*

3. *For Dr. Dekant, what is the number of rats used in individual experiments?*

(Plotzke) There is a lot of published data on the persistence of these substances. About water, with particularly D4 you have simple hydrolysis that happens in water. The ring structure opens up and breaks down into more water soluble materials and there may be more hydrolysis at different pH levels. Clay catalysis is what breaks down the cyclic materials in the soil. It happens faster in drier soil. When soil is moist cVMS moves into the air. There is also data on the air degradation by hydroxyl radicals. We are currently looking at other mechanisms of degradation, but is rapid in the air and it is broken down into water soluble substances. Where you see more persistence is in sediment. Therefore, you need to know about the concentrations of the parent material and whether there is potential for adverse outcomes associated with this compartment this can easily be assessed with a risk assessment approach. About metabolism, the processes are similar in that you get break down into water soluble substances that are excreted and should eventually degrade to silica and $CO_2$.

(Maecker) Laboratory studies are done at a pH of seven for regulatory purposes, but a study found that the average pH of surface water is 7.9 in Europe. Hydrolysis rates happen really fast and are not temperature dependent.

(Plotzke) About human health, I think Professor Dekant answered this at the end. Looking back at the lung effects, it is important to point out that a number of authorities looked at the robust data such as Health Canada, Cosmetic Ingredient Review Board, and the UK Health Safety Executive. When they apply a risk assessment approach it shows there is no risk to human health. It is important to remember that GHS is hazard-based. Sometimes we have to make decisions about GHS before we have mode of action data looking at relevance. If we knew what we know today, even D4 would not be classified. Australia has done a review on human health and has stated that there is no risk to human health in how these substances are used.

(Dekant) The issue is that you have Australia, the UK, and the US on one side and Europe on the other side that is more conservative. The Anglo-Saxons are more risk-focused. That there is no risk is well-supported by the evidence. There is no mode of action that supports genotoxicity. When EFSA looks at that, they probably will also conclude that there is no risk under present applications.

(Solomon) Assessments are done by Environment Canada and Health Canada, and they never talk to each other except through an intermediary. The conclusion was that risk is minimum, so as a board we were instructed not to look at human health issues.

(Plotzke) One additional point testing, it is important to look at how we do toxicology studies for human health or even terrestrial organisms. We are required to expose organisms to the highest possible concentrations. From a terrestrial perspective it is important to understand the difference between what is being done in the laboratory and how organisms in an environment will be exposed. You will not get an inhalation exposure of 500 ppm or 700 ppm terrestrially or environmentally. We can look at the kinetics and see that at high concentration we are in a non-linear range of kinetics. Therefore, the same effects will not be seen at the lower doses found in the environment.

(Dekant) Rodent data is quite good as experimental support of the kinetic model. Predictions are supported by measurements of the repeated inhalation by rats.

4. *Talking about assessment methods, what is the recommended method for testing cyclics? Is BMF good for assessing cyclics?*

(Solomon) To have adverse effects you must have exposure at levels that would cause adverse effects. If the bioconcentration due to lack of exposure is negligible or very small you cannot get reactions that cause events. If you do not see toxicity at the maximum concentration that you can achieve in the matrix then bioaccumulation or biomagnification is irrelevant.

(Plotzke) About biomagnification factors, I know we cannot do trophic magnification studies in the field on every chemical, so we need another way to understand the behavior of how biomagnification happens in the field. For these substances, one way to do that is metabolism and looking at the metabolic transformation rates in different organisms.

(Hashizume) I agree. For cVMSs BMF studies are more relevant than BCF studies. For cVMSs we have field data, TMF data, and in vitro data. We should combine data to find out what we know.

(Plotzke) We can also learn from this dataset about what happens in the field to inform how we better understand biomagnification for other chemicals. It is quite difficult to analyze these substances due to potential for contamination. You need to be extremely careful on the analysis side. If you use a silicone-based column then you could create misleading final data. Talking about what is going on with air and using an absorption matrix to capture the molecules, sometimes D5 on that absorbent will break down into D4 and D3. You need to make sure this is not happening in order to record the correct levels of each substance.

(Horii) We are using many siloxanes in our lives, so we need to pay attention when analyzing those compounds. If we have those compounds on our body then concentrations in the indoor air in the laboratory will increase. We need to use a simple and quick method to measure the compounds. In our lab we always use fresh solvents. That kind of attention is important.

(Solomon) The people at Environment Canada were trying to get measurements of D5 in the environment and had extreme problems. They had to set up a clean room to do so. Anyone using cosmetics or hair treatments were banned from the facility. When your blanks are higher than environmental readings then there is problem. A paper on siloxanes in the Antarctic showed that many mistakes were made while taking measurements. The quality score they got was 0.05 out of four because they made all the mistakes possible, yet it was published in a peer-reviewed journal.

(Dekant) The general issue is that with trace analysis you have to be extremely careful especially in chemicals that are widely used.

(Mihaich) That means we have to be careful when reading peer-reviewed papers. When you dissect papers and find mistakes, we as scientists need work with people in the field to understand what is really going on.

5. *With the absence of metallic ions, does it affect absorption in the human body? If siloxanes have chelation with metallic ions, what is impact on the absorption in the skin?*

(Plotzke) About the dermal absorption data, we see that regardless of the formulation (D4/D5) it stays stable on the skin. Most of it volatilizes off the skin as parent D4 or D5, so there are no reactions on the skin. What influences the penetration is the ability to volatilize.

(Dekant) Volatility is important. The chemistry does not support complex formation because there are no sites for chelation. The material volatilizes and absorption is very low.

(Plotzke) Absorption is 0.1% for D4 and even less for D5.

(Solomon) Human skin is very wet and these materials are not soluble in water, so we would not expect them to penetrate the skin. I have not seen data on insects, but that would be interesting because they have lipophilic exterior membranes.

6. *About decomposition, one specific characteristics of silicone is resistance to oxidation, ozone, or UV exposure. Therefore, how can it be that D4 or D5 decompose easily in the atmosphere?*

(Plotzke) D4 and D5 are the small monomers that make up the long-lasting and stable polymers that resist degradation. D4 and D5 can interact, but the polymer behavior is very different.

(Maecker) Reactions in polymerized compounds are very different. With respect to the half-lives of D4, D5, and D6, they degrade relatively fast compared to what you would expect if only the hydroxyl radical mechanism was at work. This implies there are more mechanisms that break them down. We need to find those mechanisms.

(Solomon) I agree that the molecule in the air is because of volatility, but on a surface of a

product there would be a smaller probability of reactions by hydroxyl radicals, which makes it more stable.

7. *You suggested CAR-induced CYP 1/CYP 2B, but I think there is crosstalk of the gene expression. There may be indirect effects due to this crosstalk.*

(Dekant) There is fairly good characterization about which P450 activities are induced, so this is the major pathway. There is always overlap of P450 activities, but the major point is that P450 induction is very similar to phenobarbital. Second, there are liver weight increases without any histopathology which indicates events are not adverse. As for interactions, you have to have fairly high concentrations, which means when you do drug interactions the blood levels of the drugs are at least micrograms or milligrams per liter, so there are specific interactions possible depending on the cytochrome P450 is blocked or induced. There is no evidence that changes in the biotransformation of another agent occurs. Even if you have agents with high environmental exposure higher than D4 and D5 it is difficult to see anything.

(Plotzke) Are you referring to cross talk between nuclear receptors? We compared it to phenobarbital, and specifically looked at CAR and PXR interaction. Even though you have liver enlargement and some similarity to phenobarbital, you do not have liver tumors after even two years.

8. *Regarding regulatory status, in Europe there may be restrictions on wash off for personal care applications. What is your forecast regarding this?*

(Maecker) The wash off personal care restriction is decided by the European Commission. There needs to be publication in the official European journal and then a 24-month phase out compliance period. After that no products are allowed to have more than 0.1% of D4 or D5. It was foreseen that this goes together with a monitoring program of the waterways on the influence on water treatment plants to see if that restriction is effective. If not effective, what other additional measures that could be taken would be discussed. Then all member states have to agree. Unfortunately, the European Commission asked the European Chemicals Agency to develop another form of restriction for personal care products, and they cannot deny that. The European Chemicals Agency has to look at the details, and asked all parties in Europe to provide relevant data. The next step is for the European Chemicals Agency to review this data with no time limit. They will then create an Annex-15 dossier and have a year to publish it.

As an industry we are convinced that these compounds are not PBT. Therefore, we are in constant debate with member states and authorities in Europe to help them to come to the same conclusion. At a workshop with German authorities in November we talked about this. On the first of August they announced that they would conduct a risk management option analysis (RMOA), which surprised us. However, they are starting at the level hazard assessment, so they are trying to do the PBT assessment again taking into account all of the latest information. In principle it is a good development since we can inform the process with QWoE in a structured way. It is getter clearer to people in Europe that the chemistry is different and that the evaluation process needs to be adapted.

For the PBT heuristics and how to apply standards, once a substance like D4 makes a POP listing, it does not matter what evaluations had been done in the states that are a part of the

Stockholm Convention. Member states would lose the ability to make their own decision on the matter.

9. *The weight of evidence description for PBT assessment annex was updated, so could you talk about that?*

(Maecker) There are two REFIT programs ongoing in Europe for POPs and PBTs. In principle that is not a new thing. Chemical industries can now give input and an opinion on how to assess substances. Even in annex XIII there is talk about weight of evidence in addition to numerical criteria. However, no one does it correctly, transparently, or in a repeatable way, which is a problem. Make sure there is a transparent way as described in a guideline on how to apply WoE so that everyone can apply it in the same transparent way. In Europe we are not there yet.

*10. How do you introduce a weight of evidence approach?*

(Solomon) It is not difficult. All you need is a scoring guide and to use it on studies and reports, but doing so takes longer than you think, especially for published studies. Sometimes it is extremely difficult to understand what they did. A badly-written paper can take you two days to understand and GLP studies can take an hour. Also, a QA process as you do in GLP is important to catch any mistakes. As long as it is transparent, the exact scoring system is not that important. We should get the same results regardless of scoring mechanism. Transparency is essential

Ellen talked about comparing 95$^{th}$ percentile concentrations to NOECs. When we did the sediment deterministic risk assessment, for sediment we used the 99.9$^{th}$ centile, which is very conservative. This was deliberate because we think these things are not likely to cause problems. The only reason we could do so was because the datasets were very good.

(Plotzke) It is important to look at new methodologies, how to apply them, and looking at all the data. We understand that criteria for screening are important, but you must look at additional data in a weight of evidence type of approach. The most important thing to keep in mind is to document the process, be transparent, and discuss it with other experts.

# Closing Comments

**Eriko Sakurai**
*President, Silicone Industry Association of Japan*

At first, we appreciate all the speakers, who are the leading researchers in this area globally. And thanks to the workshop attendees to make the workshop successful by your active participation. All the high quality of questions and discussions prove that this is a really hot topic for many of us. It was great to learn about the scientific processes of Weight of Evidence. We now believe more about the relevance of Weight of Evidence as a part of regulation method. I hope Dr. Solomon would be able to give us a short course on Weight of Evidence as he does at Universities in the near future. One of our important missions in Chemical Industry is the innovation. Our innovation supports the innovation at downstream industries such as Automotive, Electronics, Personal Care and more. Another important mission for us is our contribution to sustainability. We will continue to provide our knowledge to the science based chemical management.