Discussion points identified at the Dutch national PBT workshop

(RIVM, 12-12-2011)

In December 2011, RIVM organised a national workshop on PBT issues, which was attended by representatives from government, research labs, industry, and science (link to report: http://www.rivm.nl/dsresource?objectid=rivmp:119375&type=org&disposition=inline). During the final session of this workshop PBT-related issues were raised which still need to be discussed, either in a national or an international framework. In this document, the RIVM PBT-working group provides a short description of these issues. To a certain extent a comparison is made among the frameworks dealing with PBT issues, to see if there are any frameworks in which the issue has already been dealt with or where it is an issue currently being under debate. Please note that this comparison is not complete yet; input is appreciated. In those cases where the RIVM PBT-working group has a clear opinion on how this issue should be dealt with, this has also been included.

The current document is distributed as part of a Dutch PBT newsletter. All contributions of non-RIVM parties to this document are highly appreciated.

1. General Issues

a. What is the value/role of QSAR results?

Quantitative Structure Activity Relationships (QSARs) can serve as an independent control for experimental errors / anomalies / incorrect interpretations (e.g. volatilization instead of degradation in an OECD 301 screening study) and experimentally very difficult to handle substances, i.e. log K_{OW} determination for extremely liphophilic substances. QSARs can also be used for screening purposes.

QSARs can play an important role in the evaluation of mixtures, where it is not possible or feasible to provide an individual (experimental) evaluation of each component. Similarly QSARs and/or microbial metabolisation predictions can play an important role in the evaluation of degradation products / metabolites. In general, little information will be available on the identity of transformation products, and subsequently also no (experimental) PBT information will be available for the transformation products.

Under REACH, (QSARs) are used for PBT screening purposes: log K_{OW} as a screening criterion for Bioaccumulation (i.e. log K_{OW} < 4.5 is considered not B).In case of inconsistencies between QSAR estimates and experimental values, in REACH a Weight of Evidence procedure should be applied in which arguments should be given why one is preferred over the other.

> ***Key issue**: in other PBT related frameworks the Weight of Evidence approach is not (yet) explicitly advocated. This makes it difficult to take relevant QSAR predictions for PBT properties into account when also experimental data is available. Harmonisation among frameworks is necessary because different approaches might lead to different conclusions on the PBT status of the same substance. The use of QSAR predictions for screening purposes (in absence of experimental data) will pose less problems in most frameworks.*

b. Compartment of concern

The intrinsic properties of PBT, vPvB and POP substances are related to criteria which are encompassing more than one compartment. The P-criterion is defined for different compartments. In most frameworks if the criterion for one compartment is fulfilled, it is sufficient to denote the substance as (very) persistent.

Before adding a substance to the Stockholm Convention or the UNECE POP protocol a risk evaluation and a risk management evaluation are carried out, in which some attention is given to the relevant compartment(s). Reasons of concern should be provided in the final statement, but these are not necessarily related to the compartment of concern. Within REACH, exposure, and thus the relevant compartment, is considered when defining the most relevant additional tests for the risk assessment of a certain substance.

Key issue: *The P-criterion is defined for different compartments, but fulfilling the criterion in one compartment should be sufficient to denote the substance as (very) persistent. There are frameworks (i.e. veterinary drugs/ feed additives) which focus on only one specific compartment in the P-assessment. In such cases, if there is an indication that a substance can be considered P in one or more less relevant compartments, it is unclear how to take this into consideration.*

c. What do we want to protect?

The general protection goal concerning PBT, vPvB or POP substances, both reflected in REACH and the Stockholm Convention, is to protect human health and the environment. It is generally recognized that PBT, vPvB, and POP substances have intrinsic characteristics that make their long term effects difficult to predict. Therefore, these substances present a very high level of concern. They are strictly regulated under REACH, whereas the Stockholm Convention aims to eliminate or severely restrict these substances. If an authorization is granted under REACH the holder of that authorization shall ensure that the exposure is reduced to the lowest level that is technically and practically possible.

Key issue: *The goal should be to eliminate substances with PBT properties to prevent their adverse long-term effects on man and environment. This should not focus on the compartment where it is emitted to, but all compartments should be protected. However, not in all substance frameworks a PBT-assessment is included, and the consequences of a PBT assessment differ among the frameworks. While in some frameworks PBT substances are not permitted on the market, in other frameworks substances with PBT properties can still be used (for instance, for human pharmaceuticals environmental risk is not part of the benefit-risk analysis).*

d. Do we have to take degradation products / metabolites into account in a PBT assessment?

REACH is very explicit on how to assess degradation products, stating in its Amendment of March 15 2011 to Annex XIII "Criteria for the identification of PBT/vPvB substances": … (10) Since substances can have one or more constituents with PBT or vPvB properties, or can transform or degrade into products with such properties, **the identification should also take into account the PBT/vPvB properties of such constituents and transformation and/or degradation products.**

The Stockholm Treaty (on POPs) states in Annex D INFORMATION REQUIREMENTS AND SCREENING CRITERIA 1: A Party submitting a proposal to list a chemical in Annexes A, B and/or C shall identify the chemical in the manner described in subparagraph (a) and provide the information on the chemical, **and its transformation products where relevant**, ….. and therefore also takes the role of transformation products into account in its evaluation of the POP properties of a substance.

Key issue: *REACH and the Stockholm treaty explicitly require taking into account degradation products in the PBT assessment. How explicit degradation products are mentioned in other frameworks is open for commenting. One of the issues which might require harmonization among frameworks is the percentage of transformation products that would still require a (separate) PBT assessment. For instance, REACH considers any constituent above 0.1% which has PBT/vPvB properties sufficient to conclude that the whole mixture/product is a PBT/vPvB substance.*

e. Waste/recycling of articles with PBT-substances

With respect to recycling of articles that contain PBT-substances the same REACH rules are applicable as for substances in general. The information is fragmented within the REACH legislation and this makes it tricky to understand. ECHA has provided a fact sheet on waste and recovered substances in which the information has been grouped: http://echa.europa.eu/documents/10162/17224/waste_rec_en.pdf.
Next to the fact sheet also a more extensive guidance document is available:
http://www.echa.europa.eu/documents/10162/13632/waste_recovered_en.pdf.

A recycling operator receives all relevant and needed information via the safety data sheet on request or provides it when the placing recycled articles on the market themselves. When a substance is subject to regulation the recycling should be in line with the permitted applications. Questions remain with respect to emission and exposure to PBTs during the recycling or waste phase. In many frameworks the waste phase is not taken into account.

> ***Key issue:*** *The waste phase should be taken into account to assess if PBT substances applied in articles can be released to the environment at their end of their service life. It has to be noted that the issue described is relevant for a limited number of frameworks (e.g. REACH, biocides).*

2. Persistence

a. Temperature correction

In REACH it is specified that a simulation test (OECD 307/308/309) provides information on biodegradation under specified environmentally relevant conditions. The guidance states that where possible simulation studies should be conducted at environmentally relevant temperatures e.g. the temperature at which the environmental media was collected. However, it is recognized that it may not be practically possible to conduct the test at these environmental temperatures. In such cases attempts should be made to adapt the temperature as far as practically possible.

On temperature correction the REACH guidance states that there can be no systematic or universal correction factor for temperature that should be applied to higher tiered biodegradation studies. However, for persistence assessments where the B and T criterion have been met and simulation data is available for degradation at 20°C, consideration should be given whether temperature correction should be applied. This will be particularly important where the measured half-life is close to the persistence criteria. This correction, if applied, should be based on the Arhenius equation and extrapolate from 20°C to the temperature of the environmental media at the point of sampling. From other frameworks, such as Plant Protection Products, it is known that the temperature correction is sometimes applied the other way around, i.e. from an environmental relevant temperature to 20°C. In doing so it is assumed that the criteria are meant for 20°C. REACH, however, states that the criteria should reflect environmentally relevant half-lives due to degradation.

> ***Key issue:*** *In summary, REACH guidance recommends the Arhenius equation to be applied to data obtained at 20°C to correct half-lives to environmentally relevant temperatures. It should not be used the other way around to predict a half-life for 20°C.The temperature correction is not performed in a consistent way among the PBT-related frameworks.*

b. Bound residue

In a soil or water / sediment study it is often observed that a compound will not only be transformed into degradation products but will also be strongly bound to the soil or sediment matrix. This fraction can be divided into extractable, non-extractable and bound residues. Extractable residue (ER) is the fraction that is extractable using 'mild' extraction methods. This may include aqueous and cold solvent extraction using methods without excessive added energy. These residues are either freely available or only weekly adsorbed to the matrix. Non-extractable residue (NER) can only be extracted using more harsh conditions. These conditions may include solvent extraction using methods such as refluxing, microwaves or accelerated solvent extraction (ASE). These residues are strongly associated with the matrix and as such, the partitioning among the phases is very much in favor of sorption to components of the soil or sediment matrix. However, this sorption may be potentially reversible and thus the compound may still exert an effect on the environment. Bound residues (BR) cannot be released from the matrix and can be assumed to be irreversibly bound. Such residues are often indistinguishable from the natural organic material e.g. humus in soil.

It is current practice that in the soil and water/sediment simulation studies the DT50 of a substance is determined using 'mild' extraction methods. By doing so, both NER and BR are considered to have disappeared from the system. This approach might be valid if indeed these residues are not available for either degradation, are not available for uptake by organisms, and will not be remobilized when environmental conditions change. More research is needed to develop new methods to screen bioavailability of the different residue fractions to validate the current approach. There is also a need for deterministic methods that better characterise the individual mechanisms, as well as collective mechanisms. Such work would characterise the mechanism of binding between a test substance (having identified the key functional group(s) responsible for binding) and the key functional group(s) in the matrix (e.g. sand, silt, clay, organic matter and/or dissolved organic matter).

Even when this approach is considered acceptable, it is still critical that the extraction schemes are theoretically sound (based upon chemical first principles) and experimentally validated (e.g. spike recovery experiments). A sequential series of extractions involving solvents with a gradation of polarities seems to be a good approach since it is likely to recover the broadest range of parent forms and types of degradation products. It would be very useful to the assessors if the theoretical rationale behind an extraction scheme and the data supporting its proof of principle is provided. It is commonly accepted that microbial action can be involved in formation of NER. In this case, this could also be a matter of enzymatic catalysis of covalent bond formation. This can be further explored by investigating if there is BR and/or NER formation under sterile conditions. When degradation of the substance coincides with formation of bound residue then this might also indicate that the substance is used as a carbon source and is integrated into soil or sediment matrix.

> ***Key issue**: It is important to develop guidance on best practices to serve as a starting point for extracting chemicals and degradation products from soil and sediment, based upon their chemical characteristics. This should then be incorporated in the guidance for all substance frameworks.*

c. Lab versus field data – how to extrapolate data generated in a laboratory setting to field conditions?

Testing of chemicals in the laboratory is typically performed under optimal conditions with regard to either the factors that determine the persistence of a chemical or with regard to the ecological factors that warrant optimal functioning of test organisms (like pH, water hardness, temperature, etc.).

For instance, biodegradation is usually one of the most important processes determining the persistence of a chemical. Usually, biodegradation testing is performed under optimal conditions with regard to temperature, inoculum (typically: high numbers of active biomass and optimal test temperatures to warrant optimal growth of the bacteria used in biodegradation testing). In the field, however, conditions may vary drastically on both a seasonal basis and on a daily basis. Consequently, there is large variance in the factors influencing degradation (and hence: persistence) under field conditions: availability of nutrients, water content of soil (in case of soil degradation), diversity of the composition of the environmental compartments, and especially temperature, to give just a few examples.

Hence, extrapolation of degradation and toxicity data obtained in a laboratory setting to realistic (and varying) field conditions is complicated. In case of biodegradation, a first step could be to base the extrapolation on the activity of the competent biomass, whilst being aware that it is not simple to measure the metabolic activity of specific bacteria, because this activity is known to be quite variable. Secondly, information on the most important modifying factors (especially temperature and redox conditions) can be used to get a more realistic estimate of biodegradation under field conditions.

> ***Key issue**: As biodegradation is the most important removal process for many organic chemicals (in some cases it is the only relevant process) from the environment, and hence determines chemical persistence, lab-field extrapolation is an important topic. It should be noted that for other degradation processes like hydrolysis and photolysis, more opportunities are available to standardize test results and extrapolate these results to realistic field conditions.*

d. Photolysis water/soil

Photodegradation in water may be an important mechanism for removal of chemical substance from the environment, particularly so for substances that resist biodegradation. Information on photodegradation is often neglected in P-assessment of chemical substances, due to lack of systematic procedures to translate such information into "half-life in water".

RIVM has reviewed scientific literature on photodegradation in water, with the purpose of finding appropriate extrapolation procedures that allow for considering photodegradation as a factor in P-assessment of chemicals, taking account of the influence of variation of pH and DOM in natural waters. Chemical substances undergo direct and indirect photolysis in the environment. Direct photolysis (breakdown upon absorption of light by a molecule) is rather well studied; mechanisms are understood. Standard measurement procedures are available to determine direct photolysis rate constants in clear water. Clear-water direct photolysis rates can be extrapolated to real-water rates by systematic algorithms. Direct photolysis in water has been successfully modeled since the 1970s. Indirect photolysis (sensitization through light absorption by other compounds, followed by energy transfer to the chemical, or oxidation by photolytically formed reactive intermediates) is documented to be important for many chemicals. Mechanisms of indirect photolysis are not nearly as well understood as mechanisms of direct photolysis, however. No standard procedures are available for measurement of rates of indirect photolysis. No systematic extrapolation rules are available to adjust empirical knowledge to variable field conditions.

Available algorithms for extrapolation of measured clear-water direct photolysis rates to field conditions have been implemented in the multimedia environmental fate model SimpleBox. Using the adjusted model, example calculations have been made for a number of chemical substances to be considered candidate PBT-substances. Influence of photo degradation on persistence has been studied by including or excluding photo degradation information. Due to light absorption in natural water, photodegradation in the environment is much slower than photo degradation measured in "optically thin" laboratory water: ~30 times slower in typical fresh waters, ~400 times slower in typical coastal sea water and ~500 times slower in ocean water.

> ***Key issue**: Contribution of photodegradation in water to degradation in the environment is significant only for substances that reside in water to a considerable extent. Many chemicals do not reside in water, but in sediment and soil. It is recommended to determine the "half-life in water" as the weighted average of half-lives in the various waters typically used in environmental risk assessment, using the modeled steady-state mass fractions as weighting factors.*

e. What is the value/role of a ready biodegradability test versus the OECD 308/309 simulation studies?

A ready biodegradability test (the OECD 301 series of test guidelines) is a laboratory test which aims to prove that a substance would be readily removed in a sewage treatment plant, under experimental conditions which are highly limiting the biodegradation process (i.e. the test substance is the only source of carbon for energy and growth of the microbial community, sludge concentration is relatively low in comparison to a sewage treatment plant, high test substance concentrations, sewage is unadapted to the test substance etc.). The test design is such that a positive result (ready biodegradability) is proof of extensive degradation / mineralization under sewage treatment plant conditions, whereas a negative result (absence of degradation) only indicates that the substance is potentially not biodegradable.

In general a ready biodegradable substance is therefore thought to be non-persistent in the environment and to have a half life (in the fresh water compartment) of less then 15 days. However, a non-ready biodegradable substance can still be mineralized quickly under environmental conditions, or can be completely persistent.

Results from the OECD 301 screening test series do not give quantitative information on the rate of transformation / mineralization, not in a sewage treatment plant nor in the environment. Simulation tests like the OECD 308 and 309 tests, give a quantitative indication of environmental half lives in specific environmental compartments. Therefore a simulation test result by default will always overrule a OECD 301 ready biodegradability test result. A (single) negative test result in a ready biodegradability test can be overruled by positive results from other ready biodegradability tests, or inherent (OECD 302 series) or simulation tests. The reverse, i.e. a substance that is readily biodegradable, but still gives a very long half life in a simulation test, is also possible, although this is rarely observed. Such cases might be explained by the conditions in the ready biodegradability test that are not relevant for/representative of environmental conditions. In these cases (slow degradation in a simulation test of a ready biodegradable compound) the result of the simulation test should also be leading in the PBT assessment. This issue does not differ among PBT frameworks, although guidance on how to proceed with this is not always specifically provided.

> ***Key issue:*** *Simulation test results always overrule ready biodegradability results in the assessment of persistency, independently of the scope of the framework.*

3. Bioaccumulation

a. Bioaccumulation in invertebrates

B-criteria are usually defined as BCF values resulting from bioconcentration tests. Mostly, these kind of tests are performed with fish species. However, Annex XIII of REACH clearly mentions the bioconcentration factor in aquatic species, i.e. not restricted to fish only. BCF values can be available for algae, crustaceans, mussels, fish, etc. On the other hand, the requirement for bioaccumulation testing in Annex IX of REACH adds 'preferably in fish' to the description of bioaccumulation testing in aquatic species.

This raises the question on how to deal with the differences in bioaccumulation rate among fish and invertebrates. Should these species be treated equally, or should some species have more weight in a weight of evidence approach? Some substances bioaccumulate in invertebrates, but do not further bioconcentrate or biomagnify, which results in lower concentrations at higher trophic levels. This is called 'biodilution'. Biodilution is caused by an increased capacity for biotransformation in vertebrates, especially in homeotherms (such as fish), which are at the end of the food chain.

For substances which are bioconcentrated above regulatory triggers by invertebrates but which are not bioconcentrated by vertebrates, the PBT assessment may be very difficult. A factor to take into consideration is that field BAFs and laboratory BCFs may also give contradictory results because of the additional uptake from food in the field.

> ***Key issue:*** *At present, there is no guidance on how to compare BCF-data for vertebrates and invertebrates, and how to weigh them when both are contradictionary in terms of the B-criterion. This issue is a main priority and at the moment research is ongoing to see how in the past this problem has been dealt with.*

b. Trophic level/What are the protection goals?

This issue relates to both the difference between invertebrates and fish and to the protection goals in bioaccumulation assessment, i.e. should the focus be on (top) predators only, or is the concern for high and unpredictable bioaccumulation relating to the whole food web, even if (top) predators may not accumulate the substance to a high extent. As a consequence, it is also important how tropic levels are determined.

In most cases a full picture of the food web under investigation will not be available. And even if this is available, food web relationships from one location are difficult to translate to other locations.

In the last decade trophic levels have often been determined using $^{14}N/^{15}N$ isotope ratios, which increases reliability of food web relationships. Translation of data from one location to another, however, remains very difficult. In addition, if the ultimate goal is the protection of the total environment choices will be different from those made when protection focuses on marine top predators or humans exposed via the environment. In the latter case values for fish and mussels will likely be seen as more important than values for algae or daphnids.

> ***Key issue**: The food web approach is quite extensive and can in practice only be applied case-by-case. It is unclear what is being protected, i.e. the total environment or is the focus the top-predators?*

c. Growth correction

In standard bioconcentration tests (OECD TG 305) an overall depuration is determined, which is a combination of several processes. Some of these processes result in a decrease of the substance concentration in fish, e.g. metabolism, growth, elimination via faeces, elimination via gills, etc. If fast growing fish are used, the apparent decrease of substance via growth can overshadow all other processes. Usually this is accounted for by correcting for growth, i.e. subtracting the growth rate constant from the overall elimination rate constant. In fast growing fish, however, the growth rate constant can (appear to) be larger than the overall elimination rate constant, which clearly complicates interpretation of results.

> ***Key issue**: To apply a growth correction is the golden standard, however in those cases where the growth rate constant exceeds the overall elimination constant ,interpretation of the results becomes complicated and attention should be paid on how to deal with this.*

d. Lipids

Since, for many organic substances, there is a clear relationship between the potential for bioconcentration and hydrophobicity, there is also a corresponding relationship between the lipid content of the test organism and the observed bioconcentration of lipophilic substances. Thus, to reduce the variability in test results for lipophilic substances, bioconcentration is often normalised to a 5% lipid content (based on whole body wet weight). This provides a basis from which results for different substances and/or test species can be compared with each other. The use of 5% for the lipid content is generally accepted since this represents the average lipid content of fish commonly used in the OECD test guideline 305. Only in the IMO framework currently 6% is used, but this will be adapted in the near future. The normalization to 5% lipids does not necessarily reflect environmentally realistic lipid contents, which may vary from below 1 to above 20%, but it normalizes the data to the standard laboratory BCF tests for which the B criteria were set.

> ***Key issue**: The normalization of BCF-values to 5% lipid content is the golden standard, which is a generally accepted approach.*

e. Dietary study – BCF

The revision of OECD test guideline 305 introduces a dietary exposure test next to the aquatic exposure test. This dietary exposure test yields a biomagnification factor (BMF, i.e. a ratio between the concentrations in fish and their diet) rather than a bioconcentration factor (BCF, i.e. the ration between concentrations in fish and the surrounding water column). Under certain assumptions a BCF value can be estimated from such a BMF value. However, these assumptions clearly introduce additional uncertainties in the obtained BCF value. In the REACH guidance the estimation of a BCF from dietary studies has been included for lipophilic organic substances. It should be realised that the BMF from such a dietary exposure test differs from BMF values that are estimated from field data, because in the field exposure is a combination of both dietary and water exposure, while in the dietary exposure test, exposure via water is explicitly avoided.

> ***Key issue**: The BMF value resulting from the dietary exposure bioaccumulation test can be used to estimate the BCF value. Guidance is available under REACH, which can be use for other frameworks as well.*

f. How should BMF and BCF values from experimental and field studies be weighed?

To determine bioaccumulation potential of substances, cut-off values for the BCF are defined in most regulatory frameworks. Yet in REACH it is indicated that BMF values, as well as TMF, and BAF values can be used in a weight of evidence approach. Guidance on how to do this, however, is limited. This also relates to the more fundamental question whether high accumulation from the water phase (bioconcentration) or high accumulation from food (biomagnification) is the concern for bioaccumulation or both. A substance may have a moderate to low bioconcentration factor but a significant biomagnification factor (e.g. lindane), or have a high bioconcentration factor (in mussels and other invertebrates) but complete absence of biomagnification of even biodilution (e.g. PAHs).

In general BCF values determined in the laboratory are the basis for the trigger that defines bioaccumulation potential in regulatory frameworks. Nevertheless, when BCF values are below the trigger values bioaccumulation may still occur in the environment, e.g. due to exposure via food in addition to exposure via water. Thus, 'ultimate proof' for bioaccumulation can be found in the field where biomagnification within the food chain can occur. The revised Annex XIII criteria of REACH mentions the evaluation of this type of data in the B assessment explicitly. However, there are no criteria or additional guidance on how to interpret these data.

> ***Key issue**: BMFs (experimentally derived or from field studies) can be used as input parameter for the BCF. However, it would preferred to emphasize how to deal with both type of data in a weight of evidence approach, since it is known that both types of experimental values do not necessarily correspond.*

g. What is the value of in vitro studies?

In recent years in vitro tests have been developed to determine bioaccumulation and especially metabolism potential. Further guidance is needed to interpret such test results. Most researchers/regulators hesitate to directly compare such results with legislative trigger values. It is not clear if they can or should be used as a screening only or that they can also be used in a weight of evidence approach.

> ***Key issue:** More guidance should be developed on how to deal with in vitro data in the B-assessment.*

h. BCF cut-off values

To determine bioaccumulation potential of substances, specific criteria (cut-off values) for BCF are defined in the regulatory frameworks. This approach clearly has its limitations. The BCF by definition only takes exposure via water into account, while organisms can be exposed via food as well. Nevertheless, in the REACH guidance some practical and conservative indicators (such as cut-off values of the dimensions of the molecule, extreme high log Kow etc.) are presented that are indicative of a reduced bioaccumulation potential. At present, these indicators are still subject of debate whether or not they are based on artefacts.

> ***Key issue:** Conservative indicators of reduced bioaccumulation potential are still under debate, however, under REACH they are generally accepted.*

4. Toxicity

No discussion points have been identified.