Refined Approaches for Risk Assessment of PBT/vPvB Chemicals

Technical Report No. 112

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

Refined Approaches for Risk Assessment of PBT/vPvB Chemicals

Technical Report No. 112

Brussels, October 2011

ISSN-0773-8072-112 (print)
ISSN-2079-1526-112 (online)

ECETOC TECHNICAL REPORT No. 112

© Copyright – ECETOC AISBL
European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

All rights reserved. No part of this publication may be reproduced, copied, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the copyright holder. Applications to reproduce, store, copy or translate should be made to the Secretary General. ECETOC welcomes such applications. Reference to the document, its title and summary may be copied or abstracted in data retrieval systems without subsequent reference.

The content of this document has been prepared and reviewed by experts on behalf of ECETOC with all possible care and from the available scientific information. It is provided for information only. ECETOC cannot accept any responsibility or liability and does not provide a warranty for any use or interpretation of the material contained in the publication.

Refined Approaches for Risk Assessment of PBT/vPvB Chemicals

CONTENTS

SUMMARY

Building upon a previous ECETOC report to develop a framework for the risk assessment of PBT chemicals (ECETOC, 2005a), this report reviews the scientific developments that are available to date and details of the on going research that is being carried out with the specific aim of reducing the uncertainty of risk assessments of PBT/vPvB chemicals. Several case studies have been analysed and the literature on newly developed methodologies has been reviewed.

PBT assessment is in many cases a worst-case assessment of intrinsic properties and is not always linked to exposure assessment or the likelihood that the exposure or hazard may be manifest through routine use of the chemical. A review of some recent risk assessments of PBT/vPvB and PBT/vPvB candidate substances revealed that in most cases standard modelling techniques were used to predict environmental exposure concentrations (PECs) from emission data, physico-chemical properties and fate data and compared to predicted no effects concentrations (PNECs) derived from standard toxicity testing data and application of assessment factors.

In some cases refinements were used to reduce the uncertainty in the exposure assessment. These refinements include: The use of the overall persistence calculated with models like RAIDAR[1] or The Tool[2]; the use of environmental and biomonitoring data; predictions of spatio-temporal variation in environmental concentrations using advanced fate models and use of probabilistic models; and physiologically-based pharmacokinetic (PBPK) modelling in appropriate species to support conclusions on uptake and bioaccumulation potential. For the effects assessment refinements including the use of the critical body burden approach (CBB), more detailed insights in the mode of action and the derivation of tolerable intake levels based on available toxicological and kinetic information were applied.

The review of recent methodological developments revealed that the main improvements are related to enhancements in environmental fate and effects testing, modelling and (bio)monitoring techniques.

The approach proposed in ECETOC Technical Report 98 (2005a) that advocated the use of all data used to include the PBT categorisation within environmental risk assessment is still considered a valid starting point. This aims to reduce the inherent uncertainties involved when attempting to characterise exposure and risks of these substances. ECETOC (2005a) also recommended that, due to the special fate properties of these substances, exposure estimations should be extended to areas that are remote from emission sources (PEC refinement) and to

[1] RAIDAR (Risk Assessment IDentification And Ranking) (Arnot and Mackay, 2008) is a mass balance screening level steady-state model incorporating detailed emissions, chemical fate and effect considerations. It is capable of calculating both exposure assessment factors (EAFs) and P_{ov}. RAIDAR estimates body burden of contaminants in a representative individual.

[2] The Tool (Wegmann *et al*, 2007) estimates the characteristic travel distance (CTD) of each substance in air. Body burden in the remote region is estimated by scaling the body burden in the source region by the fraction of the chemical that is transported to the remote region.

exposure through secondary poisoning (PEC_{oral} consideration) to account for food chain bioaccumulation. It also suggested that potential effects of these substances are assessed in greater detail (PNEC refinement), by considering higher tier effects data, such as chronic data, critical body burden measurements, and identifying the mode of action. Conventional risk assessment strategies as applied to non-PBTs will not necessarily take into account all of these considerations and therefore will not necessarily come to a conclusion about risk that is both comprehensive and protective for PBT/vPvB substances. vPvB substances do not fulfill the T criteria and are considered of concern due to a precautionary assumption that increasing concentrations may cause adverse effects after prolonged exposure. For these substances it is important to base the hazard assessment on information on chronic toxicity ideally supplemented with kinetic data that allow the derivation of critical body burden or internal dose information. The comparison of these values with steady state concentrations derived by refined assessment of exposure and fate of these substances, will allow a sound risk assessment including a possible no risk conclusion. Risk assessment of PBT/vPvB chemicals can also benefit from the use of probabilistic methods to better account for uncertainties within both exposure and effects assessment. By conducting a comprehensive risk assessment of PBT substances critical uses can be identified that can be used to form the basis for appropriate risk refinement and risk management strategies where the PEC:PNEC ratio is greater than 1.

Through the examples described within this report, this task force has come to the conclusion that the choice of the appropriate refinement strategy is strongly dependent on the nature of the respective chemical and its exposure characteristics. Therefore the selection of methodologies needs to be made on a case-by-case basis. Chemical space mapping could be a tool to improve decision-making on the best refinement strategy to use for a particular chemical depending on equilibrium partitioning data (octanol-air, air-water, octanol-water). It can predict the primary compartment(s) of concern. Combined with knowledge on the exposure situation this can be used as a starting point to select and prioritise the most appropriate refinement strategy. This will target subsequent empirical based work and field-based observations to address the areas of greatest uncertainty.

Exposure assessments for PBT substances have profited from recent developments in single and multimedia models and their combination with high quality monitoring data. Single media persistence models have been developed at screening and confirmatory levels, allowing individual half-lives to be derived in individual media. These refined half-lives can be used if needed with the relevant partitioning data, e.g. for chemicals with multiple environmental discharges or undergoing multimedia transport, in multimedia models to evaluate their relative importance or determine overall persistence. However, it would be important to develop improved test methods to generate more realistic biodegradation kinetics data in order to reduce uncertainty of the model results. At present there are no criteria for overall persistence, as there are for individual environmental compartments (e.g. water, air, sediment and soil).

Consequently, further scientific discussion is required to discuss the importance of overall persistence, its applicability domain, and its inclusion at a policy level.

Several models and improvements in experimental methods have also been developed or are still in development to better describe bioaccumulation and the potential to biomagnify in the food chain. A combination of both experimental and modelling data could be a promising approach for more differentiated assessments of the bioaccumulation and biomagnification potential in the relevant organisms and food chains for a respective chemical.

An environmental effects assessment for PBT/vPvB chemicals should specifically focus on longer-term tests in the environmental compartments of concern and provide a refined food chain / secondary poisoning assessment. Relevant information on the mode of action of a chemical, both from environmental and toxicological data as well as a growing database of molecular biology ('omics) information can be used in a weight-of-evidence approach to determine the mode of action and develop tailored strategies for further testing. CBB or human bioequivalent dose approaches combining information on external exposure and dose response with toxicokinetic data provide better estimates of internal safe levels and also consider possible critical metabolites.

In conclusion the risk assessment of PBT/vPvB type chemicals needs to include higher tier evaluations and refinement. The additional studies that need to be carried out and any refinement options will need to be considered on a case-by-case basis and may include:

- Measurement of half-live ranges in appropriate environmental media using improved biodegradation tests under appropriate environmentally relevant experimental conditions (e.g. temperature, duration, inoculum source and amount) and further evaluation of degradation pathways.
- Further modelling of the environmental distribution and overall multi-media fate.
- The use of physiologically-based pharmacokinetic (PBPK) modelling in appropriate species to support conclusions on uptake and bioaccumulation potential.
- Chronic effects testing on appropriate test species based on likelihood of exposure and sensitivity.
- Targeted environmental monitoring work in appropriate media (e.g. air, sewage effluent, river water, sediment, marine water and biota).
- Long-term monitoring programmes to investigate persistence and bioaccumulation potential in the field on a temporal and spatial scale.

Whilst there are a number of generic elements that can be included within such a risk assessment (e.g. high level of refinement in both exposure and effects assessment, the need for food chain assessment, monitoring of environmental concentrations along both a spatial and a

temporal scale), the detail of the strategy that would be required to adequately reduce uncertainties associated with each chemical in question needs to be considered on a case by case basis. Each high tier assessment must take into account the specific properties and use scenarios for the chemical in question to address its specific concerns. The case studies in this report can be used as examples for the targeted application of refined methodologies for the risk assessment of PBT and vPvB properties. Further research is ongoing and other areas of research have been identified that will further increase the possibilities of reducing uncertainties in risk assessment of PBT and vPvB substances.

An appropriate risk assessment, incorporating the refinement options suggested within this report, would require significantly more data to be generated in a targeted manner. This would require a significant investment to be made in research, which may include an ongoing commitment (e.g. ongoing monitoring of environmental concentration on a spatial and temporal scale).

1. INTRODUCTION

Many national or regional regulations and regional or global conventions exist that identify and prioritise substances of concern based on their hazardous properties. For example, the European Union regulation covering the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), the Canadian Environmental Protection Act (CEPA) or the United Nations Environment Programme (UNEP), Stockholm Convention on persistent organic pollutants (POPs). The criteria for identifying substances of concern under these instruments are not the same but have many similarities through cut-off values associated with a chemical's persistence (P), bioaccumulation (B) and toxicity (T).

The criteria for PBT first came on the agenda within the EU in the revision of the Technical Guidance Document (TGD) on risk assessment (EC, 2003). This document assigned criteria to identify substances of concern as PBT or very persistent and very bioaccumulative (vPvB) based on their half-lives in selected environmental media, their bioaccumulation in biota and their long-term ecotoxicity. This hazard-based approach to chemical management was argued on the basis that 'safe' environmental concentrations for such substances cannot be established with sufficient reliability due to the unacceptably high level of uncertainty associated with quantitative risk assessment, the concerns that accumulation of such substances would be practically difficult to reverse, and the need to protect pristine (marine) environments.

These concerns regarding the ability of risk assessment tools to accurately predict the environmental fate and effects of substances of concern were then reflected within the EU REACH legislation (EC, 2006), which effectively removed risk assessment as the regulatory decision making tool for substances classified as PBT or vPvB.

ECETOC (2005a) published the work of a Task Force that had been commissioned to investigate whether the concerns expressed over the inadequacies of risk assessment methodologies are valid when applied to chemicals categorised as PBT or vPvB (ECETOC, 2005a). Their Terms of Reference were to:

- Review how the existing regulatory schemes with criteria for categorising substances as PBT address the risk assessment of those chemicals;
- identify main technical and methodological issues and uncertainties for risk-based decision making of substances that are categorised as PBTs;
- develop and recommend a risk-based strategy to address substances categorised as PBTs within a regulatory setting.

The Task Force concluded that, despite the increasing emphasis on hazard-based approaches for regulatory decision making of substances categorised as PBT, risk assessment remains a valid

tool for regulatory decision making for these substances. It was recognised that, due to their hazardous properties, substances categorised as PBT would require a highly refined risk assessment that incorporates higher tier methodologies to more accurately assess their fate and effects in the environment. But, in principle, risk assessment is a tool that can be applied to any chemical, regardless of its hazardous properties.

Since the publication of ECETOC TR 98 (ECETOC, 2005a), concerns continue to be expressed regarding the move towards hazard-based regulatory decision making of chemicals. For example, this was the topic of a Society of Environmental Toxicology and Chemistry (SETAC) Pellston workshop in 2008. This workshop brought together scientists from academia, government and industry to discuss the state of the science in understanding the behaviour and potential impact of POPs and PBTs in the environment and to make recommendations to policy-makers to improve and coordinate national and international regulations on this issue.

The result of this Pellston workshop was published as a special series in Integrated Environmental Assessment and Management (IEAM, 2009). Within the introductory article to this series the authors argue that the current regulations to categorise PBT and POP substances use criteria that are based on the state of the science established in the late 1970s and early 1980s. It was argued that in the meantime, science had produced new insights into the persistence, bioaccumulation and toxicity of substances which can be used to reduce the uncertainties associated with chemical risk assessment making it a valid tool for regulatory decision making (Klečka *et al*, 2009).

In the final article in this series van Wijk *et al* (2009) concludes that:

- PBT and POP identification and evaluation of impact are more complicated than for other chemicals and remain a challenge.
- PBT and POP assessment is associated with higher uncertainty and generally requires more data.
- For some data rich PBTs and POPs, it has been clearly demonstrated that identification and assessment of impact is feasible.
- New scientific developments and techniques are able to significantly increase the certainty of the various elements of PBT and POP assessment.
- Current scientific literature provides many successful and illustrative examples that can be used as methodologies to build on.
- Applying multiple approaches for PBT and POP assessment is advisable, because it will reduce uncertainty and may increase confidence and improve the quality of decision making.

1.1 Terms of reference

This Task Force was established to review new developments in risk assessment approaches for PBT/vPvB substances with the following terms of reference:

- Review risk assessment approaches that have been applied to chemicals that may be categorised as PBT, vPvB or POP under national or international criteria.
- Review scientific advances or opinions produced since the publication of the ECETOC Technical Report 98 on Risk Assessment of PBT Chemicals (ECETOC, 2005a).
- Update the recommendations for the risk assessment of chemicals categorised as PBT or vPvB made in ECETOC Technical Report 98 based on these reviews.
- Identify the need for additional research which would further advance the science of risk assessment when applied to chemicals categorised as PBT or vPvB.

The focus for this report is the risk assessment approaches that could be applied to chemicals categorised as PBT or vPvB. Chemicals categorised as POP have been included within Chapter 3 (Example Risk Assessments for PBTs) as these chemicals tend to be more data rich, their environmental fate and effects researched to a greater extent and subject to risk-based evaluation. By their nature, all POPs can be considered as meeting PBT criteria, but not all PBTs will meet POP criteria. This is an extremely important distinction. The risk-based evaluation and research conducted on POPs have been used within this report to inform risk assessment approaches for PBT/vPvBs through the identification of higher tier methodologies that have been applied to reduce the uncertainties associated with a more environmentally realistic risk assessment.

In proposing higher tier risk assessment approaches that could be applied to PBT/vPvBs it is assumed that the chemical in question has already been categorised as PBT or vPvB within the relevant national or international regulatory scheme. This categorisation against criteria for persistence, bioaccumulation and toxicity requires a significant data set to be available. Whilst this information will be utilised within a highly refined risk assessment as proposed within this report, the information used to categorise a chemical as meeting the PBT/vPvB criteria would normally not be sufficient alone to conduct a sufficiently high tier risk assessment where the uncertainties associated with its environmental fate and effects are appropriately reduced. An appropriate risk assessment, incorporating the refinement options suggested within this report, would require significantly more data to be generated, in a targeted manner. This would require a significant investment to be made in research, which may include an ongoing commitment (e.g. ongoing monitoring of environmental concentrations).

It is also assumed within this report that any issues associated with a chemical meeting the relevant PBT or vPvB criteria have been brought to a conclusion. For example, the environmental relevance of P (or vP), B (or vB) or T criteria within different environmental compartments has been resolved.

2. EXISTING SCHEMES OF EVALUATION FOR PBT AND vPvB PROPERTIES

A number of existing regulatory schemes addressing properties of PBT/vPvB chemicals have been reviewed in ECETOC, 2005a and will not be repeated here. A recent overview of the existing regulatory approaches and criteria including those for persistent organic pollutants has been published by van Wijk *et al* (2009); see also Appendix A. The EU, while not changing the criteria, has recently published an update of Annex XIII of EC regulation 1907/2006 including some of the screening considerations mentioned before in the guidance document for REACH (ECHA, 2008a) and giving more importance on the general view and a weight of evidence (WOE) approach in deciding on the PBT and vPvB properties (EC, 2011). Additional schemes, PBT and vPvB evaluation in the risk assessment of pesticides and the environmental hazard and risk classification scheme of medicines in Sweden are summarised below in more detail as this has not been done in the previous report.

2.1 Risk assessment of PBT pesticides

In an effort to harmonise assessments of PBT/vPvB chemicals in OECD member states to facilitate mutual acceptance, the OECD Pesticide Forum issued a questionnaire to all member countries in August 1999. The purpose of the survey was to provide a clear understanding of the data and information that are used by pesticide regulators to determine the risks associated with low-dose exposure to persistent, bioaccumulative, and toxic pesticides. A summary of the results of the survey was published by OECD, 2003.

As no harmonised approach has been established in OECD up to now, an overview of some national or regional regulations is given below.

EU: In the EU, pesticides have been regulated through EU directive 91/414 until June 2011 when the new regulation (EC) 1107/2009 came into force (EC, 2009). Whereas PBT/vPvB evaluation has not been a subject in the directive 91/414, the new regulation will use the identification of a substance as PBT or vPvB as a cut-off criterion for notification. Additionally, pesticides fulfilling part of the criteria may be considered as candidates for submission. Thus the decision is purely hazard-based and a risk assessment will not be performed. Concerning the PBT and vPvB criteria themselves (1107/2009, annex 2), the pesticide regulation will rely on the same criteria as they are used under REACH.

USA: In the US specific criteria for selection of PBT and vPvB substances exist but are not used in the context of pesticide notification. Pesticides are regulated through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); the evaluation is based on a risk assessment profile. Generally concern is given to the bioaccumulation potential.

Japan: There are no official criteria for, or regulations on, PBT/vPvB substances for pesticides in Japan. Some official criteria are set on substances which persist in soil. If substances are bioaccumulative, the setting of fish Maximum Residue Limits (MRL) may have to be considered. However, it is also effected by GAP (Good Agricultural Practice) and therefore, there are no official criteria on bioaccumulative substances.

Canada: The Pest Management Regulatory Agency (PMRA) takes into account the federal Toxic Substances Management Policy (TSMP) during the review of an active ingredient. The TSMP[3] is an Environment Canada policy (PMRA falls under Health Canada). The key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. The PMRA assesses pest control products using the TSMP criteria for persistence and bioaccumulation to identify those that contain substances that are candidates for Track 1 (virtual elimination). A substance that meets all four criteria (that is persistent, bioaccumulative, toxic and primarily the result of human activity), will be targeted for virtual elimination from the environment (Track 1 substance).

The risks associated with a pest control product containing an ingredient that is a Track 1 substance, would generally be considered unacceptable and such products would not be registered.

In recent history PMRA has concluded that certain compounds do not meet the "TSMP Track 1 criteria" for bioaccumulation because there is no credible field evidence indicating the criteria for bioaccumulation (BAF $\geq$ 5000), nor is there biomagnification in biota inhabiting the areas of use. It is therefore concluded that the compound does not meet the criteria for a Track 1 substance under the TSMP. However, the compound is considered to have PBT characteristics. The PMRA is currently developing a policy for managing PBT chemicals, and will be revisiting compounds once an approach has been developed.

Also in recent history PMRA has concluded that certain compounds do meet the TSMP Track 1 criteria and were recommending the compound not to be registered.

[3] http://www.ec.gc.ca/toxiques-toxics/default.asp?lang=En&n=2A55771E-1

Table 1: Criteria for the selection of substances for Track 1

Persistence[1]		**Bioaccumulation**[3]	**Toxicity**[4]	**Predominantly anthropogenic**[5]
Medium	**Half-life**			
Air	≥ 2 days[2]	BAF ≥ 5,000 or BCF ≥ 5,000 or log K_{ow} ≥ 5.0	CEPA-toxic or CEPA-toxic equivalent	Concentration in environment largely resulting from human activity
Water	≥ 182 days			
Sediment	≥ 365 days			
Soil	≥ 182 days			

≥ greater than or equal to

1. A substance is considered persistent when the criterion is met in any one medium.
2. A substance may be considered as persistent in air if it is shown to be subject to atmospheric transport to remote regions such as the Arctic.
3. Bioaccumulation Factors (BAF) are preferred over Bioconcentration Factors (BCF); in the absence of BAF or BCF data, the octanol-water partition coefficient (log K_{ow}) may be used.
4. A substance is considered toxic if it meets or is equivalent to the definition of 'toxic' found in the *Canadian Environmental Protection Act* (CEPA), as determined through a systematic, risk-based assessment. CEPA states: "*A substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health.*"
5. On the basis of expert judgment, the concentration of the substance in an environmental medium is due largely to the quantities of the substance used or released as a result of human activity relative to contributions from natural sources. Elements and naturally occurring inorganic compounds are not candidates for virtual elimination from the environment.

Australia: The Australian criteria were developed to be used for preventing new pesticides/veterinary medicines or industrial chemicals exhibiting PBT characteristics from entering the market, and for screening of existing chemicals for further regulatory attention.

Table 2: Criteria for PBT Chemicals

Persistence	For PBT purposes a chemical is considered persistent in a particular media if its half-life in the media exceeds the following:	
	Media	**Half-life**
	Water	2 months
	Soil	6 months
	Sediment	6 months
	Air	2 days
Bioaccumulation	For PBT purposes a chemical is considered to be bioaccumulative if it has a BCF/BAF of >2000, or in its absence of any BCF/BAF measurement a log K_{ow} > 4.2.	

2.2 Environment hazard and risk classification systems in Sweden for human medicinal products

In December 2002, the Swedish Government commissioned the Medical Products Agency (MPA) to analyse the environmental profile of pharmaceuticals. One of the resulting recommendations was an environmental classification system. The intent was to educate physicians so that the most environmentally-friendly drug could be prescribed. The pharmaceutical industry offered to participate in the development of a risk-based system.

In the interim, Stockholm County Council (SCC) and the Swedish National Pharmacy, Apoteket, developed a hazard-based system using conservative PBT criteria[4].

The combined schemes are summarised below together with a discussion of the strengths and weaknesses of the system.

Stockholm County Council hazard-based classification system

The hazard index 'scores' the drug up to a nine-point maximum, divided equally among P, B, and T (Table 3). Lack of data for any of the three parameters 'penalises' by defaulting to the worstcase score of 3.

Table 3: Stockholm County Council hazard-based scoring criteria for PBT index of pharmaceuticals

Parameter	Criteria	Score
Persistence	Ready biodegradation - OECD 301 or equivalent.	Ready = 0 Not Ready = 3
Bioaccumulation	log P_{ow} <3 log P_{ow} >3	0 3
Toxicity	LC/EC/IC_{50} < 1 mg/L LC/EC/IC_{50} = 1-10 mg/L LC/EC/IC_{50} = 10-100 mg/L LC/EC/IC_{50} > 100 mg/L	Very high = 3 High = 2 Moderate = 1 Low = 0

Swedish Pharmaceutical Trade Association (LIF) Risk-based Scheme

An industry expert team proposed a risk assessment methodology (PEC/PNEC) which was adopted as the preferred method by a broad stakeholder group.

[4] http://www.janusinfo.se/v/About-the-environment-and-pharmaceuticals/?id=9930

The risk methodology is a hybrid of the European Medicines Agency's (EMA) guideline for environmental risk assessment of pharmaceuticals (EMA, 2006) and the European Commission Technical Guidance Document (TGD) on risk assessment (EC, 2003). Where documented, reductions by removal mechanisms (i.e. metabolism, wastewater treatment, biodegradation) are accounted for. Determination of the predicted no effect concentration (PNEC) closely follows TGD recommendations and includes their assessment of PBT status. Compounds that have a PEC/PNEC ratio <1, and at the same time meet the TGD definitions for PBTs and/or vPvBs, would not be considered as posing an insignificant or low risk, but rather would be indicated as having 'hazardous environmental properties'.

The output takes the form of three tiers of increasing depth; a simple statement of PEC/PNEC risk, a further description of PBT characteristics and, finally, the data used for the assessment.

Strengths and weaknesses of the SCC combined classification scheme

The greatest strength of the SCC scheme is its ambition to make environmental data transparent to stakeholders (pharmacies, physicians and patients) such that they can make a choice about the selection of medicinal products on environmental hazard and risk grounds. Another important aspect is that the system is very clear about which classifications are based on the absence of data, indicated with an asterisk on the hazard table and the use of the statement, "[Risk] Cannot be excluded" in the risk table. This encourages the marketer to attempt to reduce uncertainty by identifying or generating missing data.

The system benefits from the unique situation in Sweden in that dispensing had been State run and efficiently accounted. This was thought to be critical to accurately quantifying usage and, therefore, to determining the PEC for risk assessment.

The SCC hazard scheme aims for simplicity and therefore largely relies on screening data. For example as medicinal products need to resist degradation to be effective, they routinely fail the ready biodegradation test and, therefore, receive a maximum persistence score of three within the hazard table. In addition, the hazard score bases its bioaccumulation potential on largely log P_{ow} data and not on measured BCF data. Since most drugs are ionisable, the distribution log D_{ow} at neutral pH provides a more realistic estimation of bioaccumulation potential and log P_{ow} overestimates.

In addition, for pharmaceuticals that do metabolise, the SCC hazard scheme does not take into account any metabolism in the patient and, therefore, frequently 'conflicts' with the risk assessment findings that do so. Looking at their 2009 listing of 373 products (SCC, 2009), there are 19 (5%) that were determined to be of 'insignificant risk' by risk assessment but have high hazard scores of

8 or 9 (see example, Table 4). This paradox illustrates the importance of considering exposure in addition to hazard and that, while PBT classification can serve as an indication of the need for further consideration, a detailed risk assessment should be given priority in the decision-making process.

Table 4: Example of conflict between risk- and PBT (hazard)-based assessments for the same substance (SCC, 2009)

Substance	Risk	PBT	P	B	T	Volume in defined daily doses
Citalopram	Insignificant	9*	3	3	3*	13 415 178

* indicates that the assessment is uncertain due to lack of data.

2.3 Conclusions

Existing schemes considering the evaluation of PBT/vPvB chemicals (as summarised in Appendix A) focus on hazard identification. The definitions and criteria for persistence, bioaccumulation and toxicity vary to a certain extent between the schemes. Risk-based considerations to make chemical management decisions are only marginally used in certain schemes. The possibilities for more risk-based evaluations based on new scientific developments and refined assessment methodologies will be reviewed in the remainder of this report and may serve as a starting point for more harmonised assessments and targeted risk management decisions.

3. EXAMPLE RISK ASSESSMENTS FOR PBTS

3.1 Example PBT chemicals for which risk assessments have been performed

3.1.1 Introduction

Examples of available risk assessments for PBT, vPvB and POP substances are discussed in this chapter. POPs are a specific sub-group of PBTs. Their long-range transport potential (LRTP) is a major aspect of the risk assessment, which is addressed by monitoring of the chemical in biological tissues of top predators in remote regions.

As mentioned previously, chemicals categorised as POPs have been included as these chemicals tend to be researched more thoroughly and hence are more 'data rich'. By their nature, all POPs can be considered as meeting PBT criteria and any risk-based evaluation and research conducted on them have been used within this report to inform risk assessment approaches for PBT/vPvBs.

Various organisations have performed risk assessments on chemicals categorised as known or potential PBT, vPvB or POP. These include the former European Chemicals Bureau (ECB), Euro Chlor and the US Environmental Protection Agency (EPA). Methodologies are generally similar, both between assessments for different substances made by the same organisation, as well as for similar substances assessed by different organisations. One universal theme of risk assessments of different organisations is the exposure versus effect metric as a means of quantitatively characterising risk. One aspect of this is the commonly employed PEC/PNEC ratio (predicted environmental concentration / predicted no effect concentration). This is utilised in all ECB and Euro Chlor PBT risk assessment reports, whilst other reports show slight deviations.

In the majority of the risk assessments presented there do not appear to be obvious differences in the risk assessment methodology employed for PBT and vPvB from typical published risk assessments, as many organisations work to a standard framework or template when conducting risk assessments. The persistence, bioaccumulation and toxic properties of these substances carry with them greater uncertainties. Therefore accurate characterisation of risk can prove to be more difficult, and may render standardised risk assessment methods with a minimum set of data unsuitable. It is important that special considerations are given when assessing the risks posed by chemicals categorised as PBT/vPvB, using expert judgement to consider the substances on more of a case-by-case basis, and here lies the obvious need for refinement of current standardised risk assessment methodologies. Examples of some risk assessments, including the substance's PBT categorisation, the risk assessment methodology and the outcome of the assessment are summarised (Table 6).

Regarding the PBT categorisations, these have been cited from the ECB's online PBT Information System[5] , which provides information on existing substances that have been subject to evaluation of their PBT properties under the Interim Strategy for REACH and the ESR programme. These categorisations have been assigned by the Technical Committee of New and Existing Chemical Substances (TC NES) or an EU member state, as appropriate, and may deviate from the standard EU PBT screening criteria, depending on special factors (such as uncertainty and reliability considerations). Data presented in the PBT categorisation column may conflict with the actual categorisation assigned by the TC NES sub-group; however it is to be assumed that the aforementioned special factors are what have lead the sub-group to their conclusion. This again highlights the fact that inherent uncertainties caused by the properties of these substances mean that currently an accurate and true assessment of their potential for, and likelihood of, adverse effects can only be confidently established when all available data are considered in a more bespoke and case-by-case manner.

3.1.2 Risk assessment methodologies

ECB risk assessments incorporate the commonly used PEC/PNEC ratio as an indication of risk for a compartment. PECs calculated for all relevant environmental compartments and secondary poisoning, if applicable, are usually modelled using EUSES (EC, 2004). However in some instances where substances are rich in environmental monitoring data, PECs have instead been derived based on these measured data by using a representative value (such as 90^{th} percentile) from monitoring databases, e.g. hexachlorobenzene (see Table 6). One issue with PBTs/vPvBs/POPs, particularly new and candidate substances, is that data on environmental concentrations of these substances are often scarce. Therefore, it is not usually an option to use monitoring data as the sole estimator of PEC. Instead the EUSES model is used, with any available monitoring data incorporated as supplementary information in order to validate the PECs generated. This was the case in all ECB risk assessment reports of PBT/vPvB/POP substances that were reviewed for this chapter.

EUSES is a useful screening level exposure and risk assessment model, which incorporates the environmental releases, as well as the distribution and fate properties of the substance. This produces a separate exposure concentration for each production and processing scenario, in each environmental compartment, which allows risk to be assessed for each of these scenarios, resulting in a clearer understanding of which scenarios are the more significant contributors to risk.

PNECs are calculated based on the TGD (EC, 2003), derived either directly from applying an assessment factor (based on the amount of reliable toxicity data available) to the lowest compartmental toxicity value, or through the equilibrium partitioning method (Table 6), which

[5] http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=pbt

can be used to estimate a soil or sediment PNEC for neutrally charged chemicals based on the $PNEC_{aquatic}$ and knowledge of some of the substance's physico-chemical properties. (An ECETOC Task Force on environmental risk assessment for ionisable organics is expected to report in late 2011 which will extend the equilibrium partitioning approach for ionisable compounds). Obviously, a PNEC derived directly from measured data for a specific compartment is preferable, but for many chemicals the equilibrium partitioning method allows a reasonably confident PNEC to be derived in the absence of these data.

The PEC/PNEC ratios are calculated for each production and process scenario, for each compartment, resulting in a large quantity of values, each of which is an indicator of risk. These values are then allocated to one of three concluding risk categories, given below. This form of risk assessment is quite comprehensive and helps in targeting specific industrial scenarios for more effective risk mitigation.

i) There is a need for further information and/or testing.
ii) There is, at present, no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.
iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Risk assessments performed by Euro Chlor include the substances hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) and differ from those of ECB in their exposure assessments. The Euro Chlor assessments are typically region- and compartment-specific. For example, those which are summarised in Table 6 are for the North Sea marine and estuarine, water and sediment, compartments (Euro Chlor, 2002a,b). The PECs in these risk assessments are based entirely on measured data, taking the 50^{th} and 90^{th} percentile as typical and 'worst case' values respectively, and are not production / process specific. Their risk characterisations are therefore broader than ECB counterparts and risk conclusions for a compartment are drawn from a single PEC/PNEC ratio. PNEC values are derived in the same way as in ECB risk assessments. The main implication of these assessments is that no attempt to assess risk beyond the predefined areas is made, and extrapolations to other locations may not be possible.

Reregistration Eligibility Decision (RED) documents have been prepared for several PBT/vPvB/POP substances, including dicofol, endosulfan and lindane (US EPA, 1998; 2002a,b). Produced by the US Environmental Protection Agency, they tend to employ the risk quotient method, comparing the ratio of estimated environmental concentrations (EECs)[6] and toxicity endpoints (LC_{50}, EC_{50}, NOEC) to various levels of comparison (LOCs) (Table 5). The EECs are modelled, based on the uses, fate and exposure routes of the substance, and the method / model by which they are derived varies. The LOCs give an indication of how broad and severe the

[6] http://www.epa.gov/opp00001/reregistration/status_page_d.htm

effects are likely to be at the current level of risk, as well as whether the risk can be mitigated through restricted use of the substance.

Table 5: Office of Pesticides Program's levels of comparison

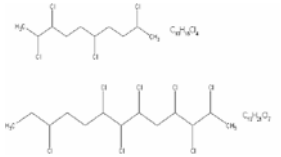
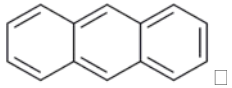
IF...	THEN the Agency presumes...
Mammals and Birds	
The acute RQ > LOC of 0.5	Acute risk
The acute RQ >LOC of 0.2	Risk that may be mitigated through restricted use
The acute RQ > LOC of 0.1	Acute effects may occur in endangered species
The chronic RQ > LOC of 1	Chronic risk and chronic effects may occur in endangered species
Fish and Aquatic Invertebrates	
The acute RQ > LOC of 0.5	Acute risk
The acute RQ > LOC of 0.1	Risk that may be mitigated through restricted use
The acute RQ >LOC of 0.05	Acute effects may occur in endangered species
The chronic RQ > LOC of 1	Chronic risk and chronic effects may occur in endangered species

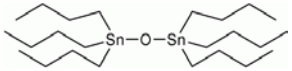
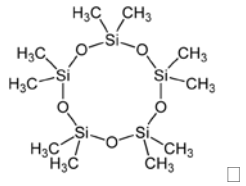
The Margin of Exposure (MOE) method has been used to assess the risk of tributyltin (TBT) compounds in specific areas (Horiguchi *et al*, 2006; Yamamoto *et al*, 2009). As with the risk quotient method, it does not involve the derivation of a PNEC. Instead, the toxicity value becomes the numerator in the equation (MOE = NOAEC/EEC) and the resulting value is compared to an uncertainty factor (UF), dictated by the amount, variety and reliability of toxicity data available. If the uncertainty factor is exceeded by the MOE then the risk is deemed acceptable.

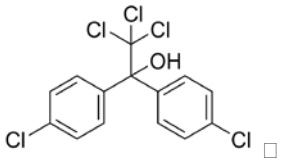
3.1.3 PBT risk assessment examples

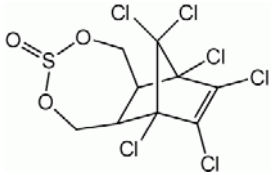
The following section presents the PBT/vPvB categorisation and risk assessment methodologies and conclusions for several substances that have been selected for evaluation of their PBT properties under the interim strategy for REACH and the Existing Substances Regulation (ESR) programme, and where published risk assessments were available. These interpretations / conclusions are summarised in Table 6.

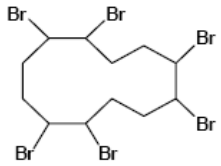
Table 6: Summaries of PBT risk assessments, including PBT categorisation, the risk assessment methodology and assessment outcome

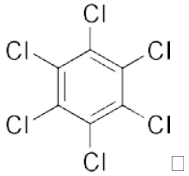
Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Alkanes, C_{10-13}, Chloro- (short chain chlorinated paraffins) 	**PBT** Half-life in sediment = 1630 days (freshwater) and 450 days (marine). Newer data indicate biodegradability in an enhanced ready test with a 50% chlorinated product. BCF up to 7816 and 40900 in fish and mollusc, respectively. BAF fish > 5000. NOEC (*D. magna*) = 0.005 mg/L. NOEC (marine mussels) = 0.0093 mg/L.	ECB, 2000; 2007	PEC/PNEC method, followed by ECB compartment and process-specific risk categories (i, ii, and iii). PECs derived by EUSES model and checked against measured data collected from the field. PNECs derived by either applying an appropriate assessment factor to the lowest effect concentration for each compartment, as per the ECHA guidance, or (if toxicity data is unavailable) using the equilibrium partitioning method.	**High priority** Unacceptable risk identified for fresh- and marine-water compartments and through secondary poisoning for some applications (e.g. metal working fluid and leather liquoring). Further testing required for sediment and terrestrial compartments, as well as for secondary poisoning.
Anthracene 	**PBT and vPvB** Biodegradation studies show low biodegradability of anthracene in fresh water (1.9% in OECD 301C), however, enhanced biodegradation due to microbial adaptation has been reported. Half-life in sediment = 210 days Half-life in soil = 79 years (vP) BCF values up to 6000 for fish. NOEC (*D. magna* without UV exposure) = 0.0022 mg/L	Greece Data from coal tar pitch RA done by the Netherlands. ECB 2008a,d	PEC/PNEC method as described in the TGD to assess risks of anthracene to water, sediments, soil and predators.	Risk associated to the presence of pure anthracene in the environment is very low due to low tonnage used. However, diffuse sources not taken into account in the risk assessment.

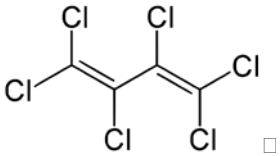
Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Bis(tributyltin)oxide (TBTO)  CAS# [25637-99-4] CAS# [3194-55-6] α-HBCD: CAS# [134237-50-6] β-HBCD: CAS# [134237-51-7] γ-HBCD: CAS# [134237-52-8]	**PBT** Half-life$_{water}$ = 4-225 days Half-life$_{sediment}$ = 1-15 years BCF = 3600 (*R. ercodes*) and 11000 (*P. major*). NOEC = 0.001 µg/L (*N. lapillus*)	Horiguchi *et al*, 2006 Yamamoto *et al*, 2009 Klingmüller and Watermann, 2003 ECB, 2008e	Margin of exposure (MOE) method. MOE = NOAEC/EEC EECs calculated by a three-dimensional hydrodynamic, ecosystem and chemical fate model (which allowed spatio-temporal changes in TBT concentrations to be predicted). An MOE greater than the uncertainty factor (UF, determined by the amount, variety and reliability of toxicity data available) indicates an acceptable level of risk.	Risk in both studies had reduced to an acceptable level over the period between the EECs, more than a decade apart. This was due to reductions in commercial TBTO use and a substantial recovery time.
Decamethylcyclopentasiloxane (D5) 	**Potential vPvB** Lack of biodegradation in laboratory tests and the relatively slow rate of hydrolysis at pHs around 7 mean D5 meets the screening criteria for P and vP criteria for water. Due to high volatility the compartment half-life approach to classifying Persistence may not be relevant for D5. Meets criteria for vB due to a BCF of D5 in fish of 7060 l/kg. Does not meet the criteria for T due to a lack of toxicity to aquatic organisms when tested at concentrations up to its water solubility limit. Further testing on long-term effects needed.	United Kingdom Environment Agency (Brooke *et al*, 2009)	PEC/PNEC method using information available as described in the TGD to assess risks to water, sediments, soil and predators from known use of D5.	No risks are identified to the air, water, and the terrestrial compartments, nor to humans exposed via the environment from the production and all uses of D5. No risks are identified to predators from the production and all uses of D5 in the UK. Uncertainties are associated with the assessment for predators because of the biomagnifications factors (BMFs) and predicted no effect concentrations used. Further information is required in order to reduce these uncertainties. Risks are identified to freshwater sediments from certain life-cycle stages of D5 using the information available. Further information is required to reduce uncertainties associated with this assessment. Industry is undertaking a voluntary test programme to address some of these issues, the results of which will be useful to refine the assessments.

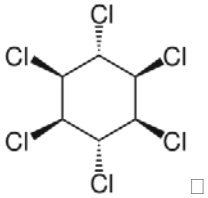
Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Dicofol 	**POP** Dicofol exists as a mixture of two isomers, p,p'- and o,p'-dicofol. p,p'-dicofol is considerably more persistent than the o,p'- isomer. The persistence of dicofol is also pH dependent – under acidic conditions the substance becomes more recalcitrant. At pH 5 the removal half-lives of p,p'-dicofol are 85.4 days in water and ca. 50 days in soil. No data are available for degradation in sediments. Hence, whilst overall the substance is not considered to meet the criteria to be categorised as persistent, under acidic conditions, one of its isomers does in fact exceed the cut off. Dicofol meets the criterion for bioaccumulation of 5000. Moderately toxic to mammals, not carcinogenic. Very toxic for the aquatic environment both in respect to acute and chronic toxicity. Dicofol meets the criterion for long-range atmospheric transport, since its vapour pressure is lower than 1000 Pa and its residence time in air is >2 days. (One isomer of dicofol has properties which, under acidic conditions, meet EU PBT criteria, however the TC NES subgroup have not deemed this to be sufficient to conclude that dicofol is a PBT overall.)	The Netherlands Royal Haskoning Environment, 2005 US EPA, 1998	Risk quotient (RQ) method. Compared estimated environmental concentrations (EECs) to toxicity endpoints. EECs were crop specific, based on environmental fate and use data and, in the case of terrestrial toxicity, the feeding rates of birds. Risk quotients were calculated and compared to the Office of Pesticide Program's (OPP's) levels of comparison (LOCs).	The RED concluded that the risk to aquatic organisms was high, since many of the crops for which dicofol is in use are in close proximity to bodies of water. All RQs for direct contamination exceeded the LOC for fresh and saltwater fish, invertebrates and shellfish. For indirect contamination all application rates exceed the LOC for shellfish. Above certain application rates (which vary depending on the crop) various other LOCs will be exceeded. One important concern from these findings is the excess of the LOC for endangered species, and this will need some consideration. According to the data reviewed under acidic pH one isomer of dicofol will meet EU PBT criteria. Little temporal monitoring data is present for the substance, dicofol. It binds strongly to soils and so will tend to not leach through to ground water, hence the only means by which it contaminates aquatic areas is through spray drift. It is still very toxic to various aquatic organisms, and there may be a requirement for further studies to assess whether greater restrictions need to be imposed. In particular at low pH values higher levels of the unbound dicofol will be available.

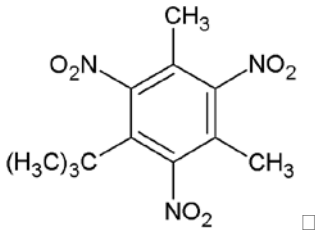
Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Endosulfan 	**PBT** Half-life$_{soil}$ = three to nine months (β-isomer) BCF$_{fish}$ = 2400-11000 NOEC$_{freshwater}$ = 0.11 and 0.07 µg/L (fish and invertebrates) NOEC$_{marine\text{-}water}$ = 0.1 and 0.45 µg/L (fish and invertebrates). **POP** Endosulfan meets the criterion for long-range atmospheric transport.	United States EPA, 2002a	Risk quotient (RQ) method. Compared estimated environmental concentrations (EECs) to toxicity endpoints. EECs were crop specific, based on environmental fate and use data. Risk quotients were calculated and compared to the Office of Pesticide Program's (OPP's) levels of comparison (LOCs). Probabilistic assessment based on actual reported applications using a 300-ft spray-drift buffer. Compared a range of EECs from models to a range of LC_{50} values.	In risk assessments for both terrestrial and aquatic compartments all LOCs were exceeded for some exposure scenarios at current application rates. In the probabilistic assessment of the aquatic compartment, calculations based on high exposure uses resulted in a 90% probability that 60% of aquatic species would be adversely affected and a 10% probability that 90% would be adversely affected. 41 aquatic and two avian species were classed as being in jeopardy.

Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Hexabromocyclododecane  CAS# [25637-99-4] CAS# [3194-55-6] α-HBCD: CAS# [134237-50-6] β-HBCD: CAS# [134237-51-7] γ-HBCD: CAS# [134237-52-8]	**PBT** HBCD does not unequivocally fulfil the P-criterion, but was considered to be "P", because of its presence in remote areas like the Arctic without known point sources. BCF = 18 100 calculated from a 32-day fish bioconcentration test. High potential for bioaccumulation supported by results from a biomagnification test and measured concentrations in wildlife. 21-day *Daphnia* NOEC 3.1 µg/L. **POP** HBCD is considered for inclusion at UNECE (Protocol on POPs under the Convention on Long-range Transboundary Air Pollution) and UNEP (United Nations Stockholm Convention on POPs) level, following submission of a dossier by Norway in 2008 (UNEP, 2010).	ECB, 2008c ECHA, 2008c UNEP, 2010	**EU RAR** The EU risk assessment, which was concluded in 2008, was carried out in accordance with Council Regulation (EEC) 793/93, using the PEC/PNEC method. The EU RAR concluded that HBCD meets the PBT-criteria as outlined in the TGD (EC, 2003). **Review Arnot *et al* (2009, 2011)** In the light of the recent POP discussions, Arnot *et al* prepared a critical review of HBCD for its POP properties and the potential for adverse effects in the environment. The following methods were used: • Environmental concentrations: Determination using biomonitoring and modelling data • Tolerable daily intake based on all available data (TDI) • Body or tissue residues	**EU RAR** • Risks identified for water, marine water, STP and terrestrial compartments related to some uses. No risks identified for air. • An overall conclusion of risk was drawn for secondary poisoning based on the fact that HBCD was considered to fulfil the PBT criteria. • Regarding human health, no risks identified for consumers, human exposed via the environment and based on physico-chemical properties. Some risks identified for workers handling the (fine) powder in production and/or industrial use. **Review Arnot *et al* (2009, 2011)** For all three methods calculated RCR's are well below 1. Only for Arctic birds using Tier 2 assessment endpoints in the body residue method, some current exposures are near NOELs associated with certain effects in mammals. When comparing the results of these three methods with the RCRs for listed POPs, Tier 1 RCRs for HBCD are approximately 1.5-3.5 orders of magnitude lower than those for listed POPs.

Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Hexachlorobenzene (HCB) 	**PBT** Half-life$_{soil}$ =2.7-22 years Half-life$_{water}$ > 2.7 years BCF (representative of low order biota) = 18621 NOECs below 10 µg/l have been observed for HCB. NOEC = 0.017 mg/L (*D. magna*) (above predicted water solubility limit). **POP** HCB meets the criterion for long-range atmospheric transport. Besides, high HCB concentrations have been found far from source regions.	Euro Chlor (North Sea), 2002b Peters *et al*, 2009 (Arctic food chain)	**North sea:** PEC/PNEC method as described in the TGD to assess risks of HCB to water, sediments, soil and predators. PEC$_{water}$ calculated by taking the 90th percentile of collected monitoring data (8 ng/L, representing a worst case). PEC$_{sediment}$ derived similarly. PNEC$_{aquatic}$ = 0.37 µg/L. Derived by applying assessment factor of 10 to the lowest reliable NOEC, based on the assumption that freshwater and marine toxicity would not differ greatly. PNEC$_{sediment}$ = 0.84 mg/kg dry weight. Generated by applying an assessment factor of 100 to the lowest reliable NOEC. *Alternative method*: PEC calculated from monitoring data, compared to Critical Body Burdens for fish calculated from BCF. CBB = NOEC x BCF(worst case) = 7.55 mg/kg ww This value was compared to measured concentrations of HCBD in fish tissues (typically around 1 to 3 ng/g ww) to assess risk. Estimated daily intake (EDI) vs PNEC$_{oral/food}$ for assessment of risk to fish-eating predators. EDI = PECfish x feeding rate (FR) **Arctic food chain:** *Alternative method*: PEC calculated from monitoring data, compared to Critical Body Burdens for predators calculated from BCF/BMF.	**North sea:** Whilst HCB possesses properties which meet EU PBT criteria, the calculations performed suggest that marine ecological risk posed to the North Sea region by the amount released to the environment is acceptable. Agricultural use of HCB as a seed dressing and fungicide has virtually ceased in Europe and the US and present day releases are low. Environmental concentrations of HCB continue to show a decreasing trend with time (Bailey, 2001) so further reduction in the risk of adverse effects in marine wild life is anticipated. **Arctic food chain:** Risk assessment showed that top predators could be at risk due to exposure to HCB via the food. However, assessment highly uncertain due to lack of experimental information to properly derive PNEC.

Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Hexachlorobutadiene (HCBD) 	**PBT and vPvB** Half-life in natural water = 4-52 weeks BCF = 17000 (rainbow trout) and 29000 (oligochaete worms). LC_{50} and NOEC (*P. promelas*) = 0.09 and 0.0065 mg/L, respectively. **POP** HCBD meets the criterion for long-range atmospheric transport.	Euro Chlor (North Sea), 2002a	**PEC/PNEC** method for marine surface waters and sediments in the North Sea region. PEC_{water} values of 5 (typical) and 12 ng/L (worst case) were calculated by taking the mean and 90th percentile of collected monitoring data available for North Sea coastal and estuarine waters and for rivers which discharge to the North Sea, respectively (EU COMMPS Database, 1998). Where necessary, the exposure data are backed up with calculated concentrations based on emission models. $PNEC_{sediment}$ generated using equilibrium partitioning method. **Critical body burden** (CBB) method. CBB = NOEC x BCF = 111 mg/kg ww. This value was compared to measured concentrations of HCBD in fish tissues to assess risk. There remains a 10^7 safety margin between the highest body burden measured against the critical body burdens indicating the risk of secondary poisoning from HCBD is very small (van Wijk *et al*, 2011).	Even though HCBD is classed as a PBT, the calculations performed suggested that marine ecological risk posed to the North Sea region by the amount released to the environment is acceptable.

Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
			Estimated daily intake (EDI) vs $PNEC_{oral/food}$ for assessment of risk to fish-eating predators. $PNEC_{oral/food}$ was taken from toxicity studies involving a surrogate species e.g. rat. EDI = PEC_{fish} x feeding rate (FR) PEC_{fish} was based on biomonitoring data. FR was estimated for both mink (0.15 kg/kg bw) and eagle (0.11 kg/kg bw).	
Lindane (gamma-HCH) 	**POP** Half-life$_{soil}$ = 2.6 years BCF up to 6000 (laboratory) and 2600 (measured) NOEC = 2.9 and 54 µg/L (fish and invertebrates, respectively)	US EPA, 2002b UNEP, 2006; 2007	Risk quotient (RQ) method. Compared estimated environmental concentrations (EECs) to toxicity endpoints. EECs were crop specific, based on environmental fate and use data and, in the case of terrestrial toxicity, the feeding rates of birds. Risk quotients were calculated and compared to the Office of Pesticide Program's (OPP's) levels of comparison (LOCs).	All LOCs were exceeded for avian and mammalian species. Acute high risk, restricted use and endangered species LOCs were exceeded for aquatic organisms. Report concludes that the initial risk assessment has been hyper-conservative. For example, the model which predicts surface water EECs, the Tier I-Generic Estimated Environmental Concentrations (GENEEC) model, assumes that 100% of the lindane coated on seeds will dissociate and be able to migrate to surface water, once the seed has been planted. Therefore the agency concludes that it does not have risk concerns for aquatic species.

Substance	Categorisation	Risk assessment author	Risk assessment methodology	Outcome
Musk Xylene 	**Proposed vPvB – SVHC under REACH (Annex XIII)** The results of two biodegradation tests clearly showed no (ready) biodegradability. In an ocean die-away test, the metabolites stayed in the water phase while the parent compound musk xylene volatilised. In addition, the ratio of metabolites-parent compound was still close to one after 159 days, which shows no rapid degradation and therefore the half-life in water significantly exceeds the criterion of 60 days. Musk xylene has a log K_{ow} of 4.9. Experimental bioaccumulation studies for musk xylene in fish showed a wide range of BCFs, among which values above the vB criterion of 5,000 L/kg. Some NOECs for specific aquatic toxicity tests were found to be at or below the threshold value of 10 µg/L. However, these results were considered inconclusive with respect to the screening of Toxicity (T) for the purpose of the PBT assessment. MX is classified as Carcinogenic Category 3, although it is realised that it is a borderline case.	ECB, 2005; 2008f; 2010	PEC/PNEC method using information available as described in the TGD to assess risks to water, sediments, soil and predators from known uses of MX.	• 2005 conclusion RAR: There is a need for further information and/or testing. This conclusion is reached, because the substance is considered a PBT candidate chemical. A further PBT- testing strategy is proposed. • 2008 addendum: (Because of its vPvB status): There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. • PEC/PNECs in all compartments <1. • MX reduces in the environment to amino metabolites. • In the aquatic environment, MX metabolites PEC/PNEC, based on limited data, would appear to be <1.

3.1.3.1 Alkanes, C_{10-13}, Chloro-

The nomenclature for this substance refers to a range of commercially available mixtures of chlorinated linear alkanes (C_{10}-C_{13}) known as 'short chain chlorinated paraffins' or SCCPs. The degree of chlorination usually ranges from 40 to 70%. They are produced by four companies in the EU but current uses which include additives to sealants and flame retardants in rubbers and textiles have declined to 400 t/year (Euro Chlor, 2011).

The ECB released a risk assessment report (RAR) for the above class of compounds in 2000 (ECB, 2000), and an addendum to this RAR in 2007 (ECB, 2007). In fact, the EU risk assessment was carried out before the substance was identified as PBT. According to these documents, alkanes, C_{10-13}, chloro- fulfil the PBT criteria although it is important to note, that this substance is a mixture and the properties of the different components may vary based upon differences in carbon chain length and the degree of chlorination. The RAR follows the ECHA Technical Guidance Document (TGD) for the assessment of PBTs and for the risk assessment.

Studies have shown that SCCPs will tend to persist in the environment, though results depend heavily on the degree of chlorination. SCCPs with higher levels of chlorination tend to show far higher levels of persistence than SCCPs of lower chlorination. Additionally, for bioaccumulation, using the measured log K_{ow} values, a BAF fish model showed BAF of over 5000 for all possible congeners, indicating that the substance initially meets the criterion for bioaccumulation. Thus SCCPs meet the criteria for PBT and vPvB under REACH. They have been identified as POPs under the UNECE POP protocol, but the Stockholm Convention applying a more rigorous scientific evaluation has repeatedly failed to identify SCCPs as POPs. The current risk profile does not demonstrate that the substance is likely to pose a risk of adverse effects to human health and the environment. In addition, more recent work has challenged the persistence data on SCCPs 50% chlorination using an enhanced OECD 301 closed bottle test. Earlier tests had difficulty in finding a method appropriate to the hydrophobicity of the substance, and therefore gave conservative results (van Wijk and Presow, 2011).

According to ECB (2007), short chain chlorinated paraffins are confirmed as meeting the EU PBT criteria for a PBT substance (meeting vP, vB and T criterion). However, the European Chemicals Bureau's (ECB) Technical Committee of New and Existing chemical Substances (TC NES) sub-group who review individual chemicals for their PBT properties have assessed these chemicals, and in their expert judgement have assigned short chain chlorinated paraffins the categorisation of PBT and not vPvB. Details of their discussion and rationale on this decision are not available.

The exposure assessment of SCCPs was carried out according to the TGD (EC, 2003). The PEC values were calculated using the EUSES model, based on extensive release and use data which

were presented in the exposure assessment. PEC_{local} were calculated for each industrial process and in each environmental compartment, as well as regional and continental PECs. These values were then compared with measured data which were collected from the field, and were found to be consistent, although it should be noted that analysing SCCPs in environmental samples is extremely difficult, not the least because of the complicated composition of the mixture. Some of the field concentrations of short chain chlorinated paraffins in water were higher than the $PEC_{regional}$ (water) generated, however these methods were assumed not to take into account that the vast majority of the substance would be adsorbed onto suspended matter, and not be in the dissolved phase.

A complete 'base set' of toxicity data was assembled, i.e. there were acute and chronic toxicity data for all three trophic levels of aquatic organisms. The $PNEC_{aquatic}$ was derived by applying an assessment factor of 10 to the most sensitive NOEC (0.005 mg/L for a 21-day study on *Daphnia magna*), according to the TGD (EC, 2003). This resulted in a $PNEC_{aquatic}$ of 0.5 µg/L being assigned. For the sub-compartment of estuarine/marine water, a PNEC of 0.1 µg/L was assigned, after an assessment factor of 50 was applied to the lowest NOEC. A $PNEC_{microorganisms}$ of 6 mg/L was assigned, after an assessment factor of 100 had been applied. The equilibrium partitioning method (EPM) was utilised in the calculation of both $PNEC_{sediment}$ and $PNEC_{soil}$, and these were estimated to be 2.17 and 1.76 mg/kg ww, respectively.

A detailed risk characterisation was performed by calculating PEC/PNEC ratios for each local process, regional and continental area and for each environmental compartment. PEC/PNEC > 1 indicated a substantial risk. Conclusions of risk were made by assigning one of three concluding categories to each compartment with respect to particular use scenarios. Risk category (iii) indicating a need for limiting the risks for some uses, was found to be relevant in the freshwater and marine-water compartments, and also for secondary poisoning. Risk category (i), signifying a need for further information and/or testing, was relevant to the sediment and terrestrial compartments, as well as on the subject of secondary poisoning. This suggests that these unacceptable PEC/PNEC ratios may be the cause of conservative release estimations rather than actual unsafe activities, and may be reduced through reductions of uncertainty through additional information.

For this chemical the conventional risk assessment based on PEC/PNEC comparison identified a risk to the aquatic environment for some of its uses. The outcome of the risk assessment identified the critical uses, which were the basis for defining the most appropriate risk management strategy and refinements of the risk assessment methodology to reduce uncertainty.

3.1.3.2 Anthracene

Anthracene is a three-ring polycyclic aromatic hydrocarbon (PAH) that is generated by incomplete combustion of organic matter. It is naturally occurring in products like coal tar and creosote, and in lower concentrations (below 0.1%) in petroleum products. Anthracene has a moderately high octanol water partition coefficient (log K_{ow}) and is therefore ubiquitously found in soils and sediments, where it binds to organic matter particles. The substance is typically found in the environment as part of a complex PAH mixture (Neilson, 1999). Because of the complex nature of PAH contamination, risk assessment is usually performed using a few representative structures. Anthracene is one of the PAHs for which environmental quality standards have been derived by the US Environmental Protection Agency and the Dutch RIVM (US EPA, 2010; RIVM, 1995).

Pure anthracene is obtained from combined crystallisation and distillation of light anthracene oil, obtained in the coal tar distillation process. In the past, anthracene was used in several industrial applications but its use in the EU decreased greatly in the last decade of the twentieth century because of the cessation of the main industrial processes where it was used, the production of anthraquinone and anthracene-9-aldehyde (ECB, 2008a). At the present time, only 10% of the total coal tar use is for production of anthracene (ECB, 2008b), used mainly in the pyrotechnic industry. Other (indirect) uses of anthracene are the application of creosote in wood impregnation, which is strongly regulated in Europe and cannot be performed by consumers, thereby strongly reducing risk of exposure. Currently, the most important emission sources of anthracene (and similar PAHs) to the environment are exhaust gases from fuel use and combustion processes in carbon anode, silicon carbide, aluminium, steel and iron production plants (ECB, 2008a).

Based on its persistence, bioaccumulation and toxicity profile, the PBT working group of the European Chemicals Bureau decided that anthracene meets the PBT and the vPvB criteria (ECB, 2008d).

In Europe, a risk assessment was performed on anthracene under the Existing Substances Regulation (ECB, 2008a). The hazard section of the risk assessment relied heavily on the ecotoxicological data compiled in the coal tar pitch risk assessment carried out by the Netherlands (ECB, 2008b). The assessment was done only for the production and use of anthracene as a pure substance, no inventory of use and emissions was done for products containing anthracene. This means that diffuse sources of anthracene emissions, which are the largest pool of emissions due to the decrease industrial use of pure anthracene, were not taken into account. However, monitoring data presented in the risk assessment showed that concentrations in the water column were in many cases below detection limit. The risk assessment showed that risks associated with the production and industrial use of pure anthracene

are very low, as expected due to the low tonnages used. Risk quotients were far below 1 for the aquatic compartment, sediment and soil. No adequate data were available to do an assessment for secondary poisoning, but anthracene, like PAHs, are often metabolised by mammals, and the risk for top predators should therefore be low. In the case of anthracene, an evaluation of the contribution of diffuse sources to the emission inventory is needed in order to determine the real risk posed by the different uses of the substance. In such an assessment, the challenge is how to deal with the natural occurrence of anthracene, a confounding factor that should be taken into account.

3.1.3.3 Bis(tributyltin)oxide (TBTO)

Bis(tributyltin)oxide (TBTO) has been used as a biocide in antifouling paints and coatings, particularly on ship's hulls to prevent the growth of barnacles.

TBTO was subjected to a PBT assessment by the ECB TC NES sub-group (ECB, 2008e) who concluded that the dissociated form, hydrated tributyltin, which forms when TBTO enters aqueous solution, fulfils the PBT criteria. In seawater TBTO is understood to form mixtures of TBTCl, TBTOH, the aqueous complex ($TBTOH_2$+) and calcareous compounds.

The fate of TBT is governed by the balance of accumulation in the surface layer due to its limited solubility and density below that of water, the degradation and volatilisation kinetics from the surface layer and its binding to suspended organic matter (SPM) followed by (reversible) deposition of SPM in sediment (Ruiz *et al*, 1996). Biodegradation to less toxic compounds dibutyltin and monobutyltin has also been described as relevant in several microorganisms including phytoplankton (Ruiz *et al*, 1996).

Extensive environmental monitoring data are available for TBT and reductions in release have led to a continuous decrease of the concentrations in the environment. The distribution of TBT is mainly restricted to the immediate vicinity of harbours and docks; nearby regions mostly have levels below the detection limit. The highest concentrations are measured in harbour sediments. Sediment and water concentrations are continuously decreasing. TBT is also found in the sludge of wastewater treatment plants and can be released to soil via the agricultural use of sludge (Klingmüller and Watermann, 2003).

TBT has been shown to have specific mode of action in the environment, namely endocrine effects on water snails, *Hydrobia ulvae* or *Nucella nupilus.* They seem to be the most sensitive species and the pseudohermaphrodism (imposex – formation of additional male sex organs in female organisms) observed in this species can be used as an indicator of exposure and reversibility of exposure. It is used for effect monitoring in the OSPAR convention. The dose

response in these organisms is very steep and effects onset is observed from chronic exposure to 2 ng TBT-Sn/L. Acute effects are only observed at relatively high concentrations. A large number of studies have been conducted on different toxic effects in mammals. Endocrine effects have not been reported in *in vivo* studies to date. The lead effect in mammals is immunosuppression. TBT inhibits lymphocyte cell proliferation and leads to atrophy of lymphatic tissue. Induction of apoptosis of T-lymphocytes and NK (natural killer) cells was also reported. TBT can inhibit the local recruitment of granulocytes and monocytes in an immune mediated reaction and inhibits phagocytosis. Based on the immune toxicity a 'safe level' in the environment of 1 nM TBT has been proposed. In humans the biological half-life is only a few hours. Effects of TBT are only expected after chronic exposure, but it is stated that insufficient information on chronic toxicity is available (Klingmüller and Watermann, 2003).

The reversibility of effects in the environment has been monitored in the French Arcachon Bay. The increasing use of TBT containing antifouling paints for boats and an increase of leisure boats in the bay in the years 1977 to 1981 led to an increase in organotin concentrations in the bay water from around 1 ng Sn/L to 100 ng/L that resulted in an elimination of the local oyster cultures. Following a ban of TBT use in antifouling paints for ships in 1982 the levels decreased to 1 ng Sn/L in the late 1980s and early 1990s and a relative quick reconstitution of the oyster cultures was observed. However, other populations, like the most sensitive molluscs may have taken longer to recover (Ruiz *et al*, 1996).

Two regional risk assessments of TBT compounds incorporated the margin of exposure (MOE) to assess the risk posed for two coastal regions of Japan (Horiguchi *et al*, 2006; Yamamoto *et al*, 2009). The estimated environmental concentrations (EECs) were generated based on chemical fate models, and these were compared to environmental concentration data collected from the field. Both papers incorporate three types of model: A three-dimensional hydrodynamic model, an ecosystem model and a chemical fate model. A program (RAMBT) was developed which generated databases using the hydrodynamic and ecosystem models and then performed calculations on them using the chemical fate model. Using this combination of modelling, spatio-temporal variations in TBT concentrations could be identified, which were used to identify unacceptable levels of risk in the regions where other risk assessment methods would not. The risk posed to the Japanese short-neck clam (*Ruditapes phillipanarun*) of Tokyo Bay with respect to growth effects was assessed (Horiguchi *et al*, 2006). There were insufficient toxicity data available on this particular species, so data on a closely related species (*Mercenaria mercenaria*) were used. The NOEC value (4.1 ng/L) was generated through the analysis of a study which measured the maximum valve lengths of this species when particular concentrations of TBT were present.

MOE calculations in this risk assessment were performed for the 95-, 50- and 5-percentiles of the estimated EECs and compared with the uncertainty factor (UF) which was set at 1 because it was

assumed that the effect of TBT on growth would not differ significantly between the two species. It was found that the level of risk was high in 1990 in the winter period, but for 2000 and 2007, after the TBT levels had had a chance to fall following the restrictions placed on the use of the substance, the risk had become acceptable.

In addition, the risk to all aquatic life in the Ise Bay region of Japan has been considered (Yamamoto *et al*, 2009). A NOEC of 1 ng/L for *Crassostrea gigas* was chosen and the UF was set to 100 in concordance with US, Canadian and Japanese regulations. MOEs were calculated for 1990 and for 2008 (i.e. representing before and after the TBT use prohibitions). A high level of risk was identified at the concentrations estimated in 1990, however they had reduced substantially by 2008 and the risk was deemed to be no longer present.

In conclusion TBTO transforms into various mono-TBT compounds when dissolved in aqueous media, and these compounds fulfil PBT criteria. The environmental fate, toxicity and mode of action of TBT both for environmental organisms and humans (which is interestingly not the same) are well understood. Several lead effects and modes of action for different biota have been described, which allow a more reliable identification of safe levels. Sensitive marker organisms have also been identified. Sufficient data on monitoring, distribution and trends in environmental concentrations and biota are available to allow a risk assessment to be conducted. Risk reduction measures have already been applied and their effects have been monitored. For example TBT compounds are now prohibited for use in anti-fouling coatings for ships and, as a result, have had time for their environmental concentrations to be reduced. The two risk assessment documents available conclude that the risk posed by TBT compounds has moved to an acceptable level over the last two decades.

3.1.3.4 Decamethylcyclopentasiloxane (D5)

Decamethylcyclopentasiloxane (D5) belongs to a group of chemicals known as cyclic volatile methylsiloxanes (cVMS). A major use of cVMS fluids is in the production of silicone polymers but D5 is also used directly in personal care products, such as anti-perspirants, hair care and skin care products.

In 2009 the UK Environment Agency reported on a PBT/vPvB assessment as well as a quantitative risk assessment conducted on D5 (Brooke *et al*, 2009). This assessment was conducted with the cooperation of the Silicone industry (Centre Européen des Silicones, or CES) using information available at the time.

Based on this assessment, D5 meets the criteria of a potential vPvB substance. This assessment is based on:

- Poor biodegradability as demonstrated in ready biodegradation studies. However, the interpretation of these studies is problematic due to rapid loss of D5 in test systems due to its highly volatile nature.
- A relatively slow rate of hydrolysis at pHs around 7.
- An experimentally derived bioconcentration factor (BCF) in fish of 7060 l/kg.
- A lack of toxicity observed when tested at concentrations up to its water solubility limit.

However, the report mentions mitigating factors that need to be considered further before a final vPvB categorisation is assigned. In particular, D5 is lost from water by volatilisation to air where removal through wet and dry deposition is considered minimal and degradation occurs to increasingly water soluble silanols through hydroxyl radical substitution. The report argues: "*The current criteria for persistence are related to degradation half-lives in each individual compartment (aquatic, sediment, etc.). These may not be the most appropriate for a substance such as D5 as it is likely to be removed from the aquatic compartment more rapidly by physical process than by degradation. Thus, the overall persistence of the substance, including the potential for transport over distances and the effects at remote locations, needs to be assessed. Currently there are no criteria for this, so both further scientific discussion and consideration at a policy level are required.*"

The risks to water, sediments, soil and predators were assessed based on D5 usage information provided by the CES. The dataset available to conduct this quantitative risk assessment was reasonably complete, however gaps did exist and the report recommends a number of areas where the risk assessment would benefit for further refinement.

The overall conclusions of the risk assessment reported are:

- No risks are identified to the air, water and terrestrial compartments, nor to humans exposed via the environment from the production and use of D5.
- No risks are identified to predators from the production and uses of D5 in the UK. However, they note that scenarios at 2 sites outside the EU lead to RCRs >1 for freshwater predators.
- Uncertainties exist in the assessment of risk to predators due to the BMFs and PNECs used. These uncertainties relate to a lack of guidance on how to interpret the data available as well as a lack of input data.
- Risks are identified to freshwater sediments from some life-cycle stages of D5 based on the data available. Further data are required in order to refine the risk assessment.

Based on this risk assessment, a number of recommendations are made for further information, combined with information being generated by the CES through a voluntary test programme. This includes the following studies:

- Refinement of the sediment risk assessment through site-specific data on emissions and further toxicity testing on sediment organisms (*Lumbriculus* and *Hyalella*).
- Further evaluation of atmospheric degradation pathways.
- Degradation in a wastewater treatment plant and sludge.
- Degradation in sediment under aerobic and anaerobic conditions.
- Further modelling of the environmental distribution and overall fate.
- Further bioaccumulation information, for example using PBPK models of fish and extensions of these models in fish and mammals to other species.
- Environmental monitoring studies (including air, sewage effluent, river water, sediment and biota).
- Long-term monitoring programmes to investigate persistence and bioaccumulation potential in the field, including:
  - Time trends using freshwater and marine sediment cores from local, regional and remote locations as well as archived biota samples.
  - Spatial distributions using sediment and biota samples along transects of freshwaters from local, regional, and remote locations.
  - Marine samples (sediment and biota) from regional and remote locations.
  - Air samples from local, regional and remote locations.

In addition, a key need in the refinement of the D5 risk assessment is the availability of robust analytical techniques that adequately take into account its unusual physico-chemical properties, especially the high volatility.

3.1.3.5 Dicofol

Dicofol, (trade name, kelthane), is an organochlorine pesticide, produced either by the reaction between chloral, monochlorobenzene and oleum, or from DDT, which may be present as impurity in the product. EU directive 79/117/EEC prohibits the use and marketing of products containing less than 78% p,p'-dicofol or more than 1 g/kg of DDT or DDT related products. In the EU dicofol is produced by three companies. Information on the process is known only for the factory in Spain, where manufacture is not DDT based.

Dicofol exists as two isomers: p,p'- and o,p'-dicofol and is similar in structure to DDT. The only difference is the substitution of a hydrogen atom on DDT for a hydroxyl group on C-2 of dicofol. However, this has a dramatic effect on the difference in chemistry between the two compounds, with DDT having much lower water solubility and greater bioaccumulation potential. The metabolites of dicofol are less toxic than dicofol itself, whereas the main metabolite (DDE) of DDT is more toxic than DDT. For these reasons, the potential danger to

the environment from dicofol has been given much less attention but there is enough data available to conduct a risk assessment.

Dicofol is used as a miticidal or acaricidal spray on fruits, vegetables, flowers and field crops. It is approved for use on apples, pears, blackcurrants, hops, strawberries, and protected crops of cucumbers, tomatoes and ornamentals. Therefore, it is emitted to the air but most is deposited to soil. Laboratory studies indicate that dicofol binds strongly to soil and will not leach significantly through soils into groundwater, hence the most likely pathways are through indirect means by which it will contaminate surface water through spray drift during application and run off. Application figures are not generally available as most countries do not record usage data according to particular active ingredients. The UK data show the average amount of dicofol active ingredient used between 1994 and 1997 was 1,143 kg per year. Residues of dicofol have been recently found on imported fruits.

According to the Reregistration Eligibility Decision (RED) document for dicofol (US EPA, 1998) there is sufficient evidence to classify the p,p'-isomer of dicofol as a PBT. However, it should be noted that the half-life of dicofol will only exceed the screening criteria in acid water (pH <5). The TC NES sub-group has concluded that dicofol is a POP, but not a PBT overall.

The RED environmental risk assessment was conducted using the risk quotient method, where RQ = Exposure / Toxicity. Ecotoxicity data were compared to modelled estimated environmental concentrations (EECs) from aquatic exposure, providing a screening-level risk assessment for dicofol, at maximum application rates, for each crop upon which it was used. The data were also compared with monitoring data from crops from Florida, California and New York.

The RED concluded that the risk to aquatic organisms was high, since many of the crops for which dicofol is in use are in close proximity to bodies of water. All RQs for direct contamination exceeded the levels of comparison (LOC) for fresh and saltwater fish, invertebrates and shellfish. For indirect contamination all application rates exceed the LOC for shellfish. None of the use patterns exceed the LOC for chronic risk to fish, however above certain application rates (which vary depending on the crop) various other LOCs will be exceeded. An important finding was that LOCs were exceeded for endangered species.

The model overestimates peak environmental concentrations and does not indicate a chronic concern. There is still some concern over the acute effects however. The studies did not provide sufficient information to determine whether the reported residue concentrations reflected typical conditions, and further studies on dicofol's interactions in specific locations need to be performed. There are no monitoring data available from countries which do not allow the use of dicofol, nor is there any from remote or Arctic areas, so the knowledge of dicofol's accumulation in these regions is somewhat limited.

Since dicofol is still in commercial use overall levels of the substance in the environment will fluctuate, being restored to its highest level after each application. The average concentration is relatively constant in the exposed areas. A concern is that dicofol formulations may contain excessive amounts of DDT, which can be an impurity of its manufacture depending on the manufacturing process, and there is suspicion that there are such formulations in use in China (Yang *et al*, 2008). Most papers related to dicofol refer to it only as a contributor to DDT accumulation, holding it in much lower regard (Qiu *et al*, 2005; 2009).

Although some refinement was used in the existing assessment in particular with regard to exposure estimates from monitoring and modelling approaches, further information would be needed for a more environmentally realistic risk assessment. Little temporal monitoring data is available for dicofol. Higher concentrations of the unbound dicofol will be found at low pH values. It is very toxic to various aquatic organisms, and there may be a requirement for further studies to reduce uncertainties and adequately assess the risks.

3.1.3.6 Endosulfan

Endosulfan was developed in the early 1950s and gained USDA approval for the use of the product in the US in 1954. The range of studies on pesticides in those days was extremely limited and aspects such as the extreme toxicity of the material (to other species such as birds), its long-range transport potential and the finding that it is an endocrine disruptor were subsequently identified. It is difficult even with the knowledge of the existing data to see how it could still be retained on the market.

Endosulfan is a broad-spectrum pesticide and acaricide. It has become ubiquitous in the northern hemisphere, even in remote areas (e.g. the Arctic) where it is not in use. The EPA released a RED document on endosulfan. This contains details of the chemical risk assessment which they performed on the substance (US EPA, 2002a). From this document a PBT assessment can be carried out. The full risk assessment report does not appear to be available to the public.

Endosulfan exists as two chemically different isomers, α- and β-endosulfan. The β-isomer is known to persist in soils with half-lives above the persistence limit of PBT criteria. Under aerobic conditions β-endosulfan biodegrades with a half-life of between three and nine months. One of endosulfan's degradation products, endosulfan sulphate (from soil metabolism) is also persistent and the combined residues have an estimated half-life of between 9 months and 6 years. Endosulfan's degradation products are also of toxicological concern. β-endosulfan fulfils the vP criteria according to REACH.

According to the RED (US EPA, 2002a), the β- isomer of endosulfan fulfils the EU PBT criteria for a very persistent (vP), bioaccumulative (B), or even very bioaccumulative (vB) and toxic (T) substance. The ECB's Technical Committee of New and Existing chemical Substances (TC NES) sub-group has categorised endosulfan as PBT and POP (ECB, 2008g). The EPA risk assessment compared toxicity endpoints (LC_{50} and NOEC) to estimated environmental concentrations (EECs). The EECs were calculated based on environmental fate and use data and were generated for each particular crop for which endosulfan is used. In the risk assessment to the terrestrial environment EECs were generated based on ingestion of residues on food. Acute high risk, restricted use and endangered species LOCs are exceeded for both birds (RQ range: 0.02-0.53) and mammals (RQ range: 0.05-40). Chronic RQs also exceeded LOCs for all food items, except seeds, for birds, and for all food items for mammals.

In the risk assessment to the aquatic compartment, surface water EECs were generated using a computer model which assumed the maximum application rates (in 2002), with a 300-ft spray drift buffer. Based on this model, all LOCs (chronic and acute) were exceeded by their respective RQs, suggesting an unacceptable level of risk. Acute RQs ranged from 1.04 to 64.2 and chronic RQs from 1.5 to 704. Estuarine / marine species were approximately one order of magnitude more sensitive to endosulfan than their freshwater counterparts. In a probabilistic assessment, calculations based on the high exposure uses (e.g. tomatoes) resulted in a 90% probability that 60% of aquatic species would be adversely affected and a 10% probability that 90% would be affected. Evidence was presented to suggest that a large number of endangered species had been jeopardised by endosulfan use and a review of the Ecological Incident Information System indicated that since 1971, a total of 91 incidents have been associated with the use of endosulfan.

Based on the results of the risk assessment established using several refined methods, such as calculation of residues in food, modelled surface water concentrations and probabilistic risk assessment, specific areas for risk reduction could be defined. EPA has now concluded that endosulfan's significant risks to wildlife and agricultural workers outweigh its limited benefits to growers and consumers nationwide. The endosulfan manufacturer is in discussions with EPA to voluntarily cancel endosulfan uses. EPA is working out the details to terminate all endosulfan uses while considering growers' needs to timely move to lower-risk pest control practices.

3.1.3.7 Hexabromocyclododecane (HBCD)

Hexabromocyclododecane (HBCD) is used as a flame retardant, mainly in the building and construction industry for insulation (extruded or expanded polystyrene foam) and in textile industry. Technical or commercial HBCD (t-HBCD) is a mixture of primarily three diastereomers: α-, β-, and γ-HBCD. In technical HBCD the γ isomer is the predominant isomer (70 to 95%).

An EU risk assessment on HBCD was concluded in 2008 (ECB, 2008c). It was carried out using the PEC/PNEC method as supported by the TGD (EC, 2003). The EU RAR concluded that HBCD meets the PBT-criteria as outlined in the TGD. HBCD clearly fulfils the B-criterion (BCF = 18,100), but does not unequivocally fulfil the P-criterion, based on experimentally derived half-life values. HBCD was considered to be 'P' due to its presence in remote areas, such as the Arctic, without known point sources. HBCD was also concluded to meet the T-criteria, mainly based on the results of a 21-day *Daphnia* study, with a NOEC of 3.1 μg/L. However, in this study a co-solvent was used, and HBCD was tested in concentrations above the water solubility limit of the γ-isomer. The effects of the co-solvent used in the ecotoxicological studies were not quantified.

The validity of using aquatic exposure-based criteria greater than a substance's water solubility limit and aquatic toxicity testing in general can be questioned for substances with very low water solubility like HBCD. The available testing showed that exposure of aquatic organisms to HBCD, at levels that are at or just below the water solubility limit, resulted in no adverse effects. It is likely that the T-criterion (NOEC < 10 μg/L) is inappropriate and unsuitable for the PBT categorisation of HBCD and other substances with very low water solubility. In these cases an assessment of toxicity for more relevant compartments, such as sediment would be more appropriate.

In 2008 the Nordic Council of Ministers completed a report (TemaNord, 2008) and in 2010 the UNEP made a risk profile (UNEP, 2010) in which available data on HBCD were compared with the criteria for POP categorisation. The TemaNord report is used as a basis in the ongoing POP discussions at UNECE (Protocol on POPs under the Convention on Long-range Transboundary Air Pollution) and UNEP (United Nations Stockholm Convention on POPs) level. In the TemaNord report, HBCD was considered to meet the P-criteria. This was mainly based on temperature-adjusted degradation half-lives of α-HBCD and γ-HBCD in aerobic sediments: A method that is not recommended (Boethling *et al*, 2009; SETAC, 2008).

In the light of the POP discussions, an inter-industry working group on HBCD[7] has commissioned a critical review of all available data on HBCD, by a team of independent environmental scientists. An environmental risk assessment of HBCD has been carried out (Arnot *et al*, 2009; 2011). Screening-level risk characterisation results were compared with results for some listed POPs.

Mass balance model simulations of HBCD were compared with the results of classified POPs, substances that are not considered to be POPs (non-POPs), and substances that are presently

[7] The HBCD Industry Working Group gathers HBCD producers and users in the polystyrene insulation foam sector, the major application of HBCD. The HBCD producers are represented by EBFRIP (European Brominated Flame Retardant Industry Panel) and the HBCD users in the polystyrene insulation industry are members of PlasticsEurope (for expandable polystyrene) and Exiba (for extruded polystyrene).

under review as candidate POPs (candi-POPs). HBCD is shown to have some potential for long-range transport and overall persistence (P_{ov}); however, other non-POP chemicals also show these properties. The properties for HBCD were generally found to be lower than for POPs and candidate POPs, particularly when median and lower bound degradation half-lives for HBCD were considered. The model simulations did not provide clear evidence for assigning HBCD as a 'POP' or a 'non-POP', largely because of the uncertainties in the half-life data and the wide range of LRTP and P_{ov} values for POPs and non-POPs.

Available monitoring data found in literature were separated into general categories of 'local near-point source', 'source' and 'remote' areas. HBCD is detected in each of these regions indicating a certain potential for long-range transport. Spatial trends show decreasing concentrations with increasing distance from known point sources. Temporal trends in the monitoring data show no consistent trends. There seem to be slight increases in certain compartments over the past 15-20 years; however, other compartments show either no noticeable increase or decreasing trends.

Emissions estimates were used to model steady state concentrations in a range of representative species of varying trophic position (fish, birds, marine mammals, and humans). These predictions were compared with monitoring data from regions in Northern Europe (Sweden, Norway). Upper trophic level organisms, particularly species that consume fish such as marine mammals and piscivorous birds, were predicted to have the highest exposure concentrations. These predictions are corroborated by the available monitoring data and indicate a potential for bioaccumulation / biomagnification.

Model calculations combining fate and food chain bioaccumulation models were also used to estimate response times for HBCD in the environment. Response time is defined as the time difference between changes in emission levels and corresponding changes in concentrations in biota in particular the time to reach a steady state situation. Response times were predicted to range from a few days (air) to lower than five years in fish and humans and above five years in soil. This is relatively low compared to some listed POPs (e.g. PCB-180), which have much longer response times in the environment. This indicates that concentrations of HBCD in the environment are expected to decline faster in response to emission reductions compared to many listed POPs. The relatively short response times for HBCD also partly explain why steady state model predictions are in good agreement with monitoring data.

The classical risk assessment method comparing exposure concentrations in physical compartments of the environment with effect or estimated no-effect assessment endpoints derived from laboratory studies resulted for HBCD in an RCR of 10^{-6} to 10^{-4} in the Arctic environment. However, this is not the most recommended method for substances with a very low water solubility and high bioaccumulation potential such as HBCD. The body / tissue-residue and

tolerable daily intake (TDI) approach were applied. These two approaches are considered viable methods to estimate the likelihood of significant adverse effects in upper trophic level organisms in source and remote regions. For the TDI approach, estimated dietary exposure for Arctic biota was well below the most conservative NOEL, suggesting no potential for significant adverse effects (RCR $8.3x10^{-3}$). Body or tissue-residues related to effect levels were directly compared to organism monitoring data. This method indicated that significant adverse effects from current exposures to HBCD are unlikely for fish and marine mammals. For Arctic birds using Tier 2 assessment endpoint data some current exposures are near NOELs associated with certain effects in mammals (Arnot *et al*, 2011). Newer data on bird toxicity that are only available as abstracts up to now, suggested that a risk for birds could not be excluded. (UNEP, 2010).

When comparing the results of these methods with the RCRs for listed POPs, Tier 1 RCRs for HBCD are approximately 1.5-3.5 orders of magnitude lower than those for listed POPs. When considering all Tier 2 endpoints, HBCD RCRs are comparable to some of the listed POPs. However, as highly sensitive endpoints that are not necessarily representing adverse effects were included for HBCD, but not for other listed POPs a more comprehensive risk evaluation may be appropriate to consider the relevance and reliability of all Tier 2 endpoints.

In a separate exercise, the benchmarking technique was used effectively in the persistence and long-range transport assessment of hexabromocyclododecane (HBCD) using two models. The two models used in the benchmarking exercise are: (1) RAIDAR (Risk Assessment IDentification And Ranking) (Arnot and Mackay, 2008) and (2) The Tool (Wegmann *et al*, 2007). RAIDAR is a mass balance screening level steady-state model incorporating detailed emissions, chemical fate and effect considerations. It is capable of calculating both exposure assessment factors (EAFs) and P_{ov}. RAIDAR estimates body burden of contaminants in a representative individual. The Tool estimates the characteristic travel distance (CTD) of each substance in air. Body burden in the remote region is estimated by scaling the body burden in the source region by the fraction of the chemical that is transported to the remote region. It was decided to look further into the overall persistence (P_{ov}) and LRTP of HBCD as, when compared directly to persistence criteria, experimentally derived half-life values did not strictly fulfil the P criterion. The RAIDAR model was also used to predict an exposure assessment factor (EAF), which combines P and B elements to obtain a single value for the comparison of chemicals in terms of exposure potential. Through a combination of the results of the two models, an estimation of total body burden (TBB_U) was also possible.

The median, upper- and lower-bound degradation half-lives of HBCD were input into the model (The Tool) in order to identify the model results dependencies on these particularly uncertain parameters. The model results consist of three different parameters: Overall persistence (P_{ov}; days), characteristic travel distance (CTD; km) and transfer efficiency (TE; %). The Tool calculates these parameters for three different release scenarios: 100 % emissions to air, 100%

emissions to water and 100% emissions to soil. The largest value from each of these is then taken as the 'worst case'. P_{ov} results from the modelling illustrated a strong dependency on the chosen half-life values for HBCD. It was estimated that for the lower and median half-life (HL) assumptions the P_{ov} would be similar to that of the non-POPs. However, for the upper HL assumption the P_{ov} was more comparable to the candidate and known POPs. This demonstrates the importance of clarifying the uncertainties in the current degradation data for HBCD. CTD values were calculated to be closer to the non-POPs than known or candidate POPs for all three half-life assumptions of HBCD. In the case of TE, the lower and median HL assumptions of HBCD resulted in TE predictions in the region of the non-POPs, whereas the upper case HL was approximately an order of magnitude lower than the candidate POPs and approximately 50% lower than the TE of known POPs. These estimations illustrate that overall, long-range transport potential appears to have less of a dependency on removal half-lives.

In the EAF benchmarking exercise with the RAIDAR model the three diastereoisomers of HBCD (alpha-, beta- and gamma-HBCD) were modelled, along with the upper and lower bound half-lives of the HBCD mixture and compared again with several candidates, known and non-POPs. Level II and level III fate calculations were conducted for each case and the results were ranked in order from the highest EAF to the lowest one. The individual HBCD isomers were found to have EAFs between those of the non-POPs and the candidate/known POPs. This is indicative of HBCD having an exposure potential somewhere between the benchmark POPs and non-POPs when emissions are treated as uniform. The upper and lower bound half-life HBCD calculations resulted in rankings in the middle of the POPs (for the upper HL) and non-POPs (for the lower HL). This illustrates uncertainty in terms of the level of concern warranted for HBCD based on its compartmental degradation half-lives.

In the P_{ov} benchmarking exercise the same assumptions were applied and the different diastereoisomers of HBCD were modelled again. The P_{ov} results from RAIDAR were generally lower than those calculated using The Tool, with even the upper HL estimates for HBCD being generally well below those predicted for candidate and known POPs. It was deemed that the higher P_{ov} predicted using The Tool was due to the fact that this model does not consider degradation in sediment.

A sensitivity analysis was conducted for t-HBCD using the Level III scenario with a mode-of-entry of 50% emissions to water, 50% emissions to air. The endpoint selected was the total body burden, based on the emission rate of 1 kg h^{-1} (TBB_U; ng) for the organism of highest concern (marine mammal). From this analysis it was found that the biotransformation half-lives in fish (food source) and aquatic mammal were by far the most sensitive parameters, having contributions to variance of 0.50 and 0.42, respectively (accounting for a total of 94%). This outcome is as expected, as biotransformation half-lives are a direct determinant of the overall residence time within an organism.

A final benchmarking exercise was conducted whereby the two models were combined to compare HBCD with benchmark substances by estimating screening level exposure potential for both a 'source' and a 'remote' region. In conducting this, RAIDAR was used to estimate total body burden (TBB_U; mmol) in a species of concern (marine mammal). The Tool was used similarly to estimate characteristic travel distance (CTD; km) in air. Estimated total body burdens of the 'source' region were scaled down using the CTD calculated in The Tool to estimate body burdens likely to be found in a 'remote' region 2500 km away. From these calculations it was found that the estimated TBB_U in remote regions spanned > 20 orders of magnitude among the benchmarking chemicals. Some non-POPs were found to have higher TBB_U than the known or candidate POPs. HBCD's TBB_U was comparable to one benchmark POP (heptachlor) but several orders of magnitude lower than others. It was also approximately three orders of magnitude lower than the candidate POPs.

The various model predictions, comparisons to known candidate and non-POP benchmarks, and sensitivity analyses placed HBCD between known 'POP' and 'non-POP' chemicals. A refinement of the data on degradation half-lives as well as half-lives in biota could lead to a better model estimate and reduce uncertainties when used in a risk assessment approach. However, the model calculations indicate that relative to known POP chemicals a more rapid decrease in environmental levels can be expected. Ongoing monitoring programmes will be useful to confirm this conclusion.

3.1.3.8 Hexachlorobenzene

Hexachlorobenzene (HCB) is a chlorinated hydrocarbon that was commonly used as a fungicide for seed dressing until the 1970s. It was also used in rubber, aluminium and dye manufacturing and wood preservation activities, but the agricultural use of HCB dominated its emissions during the 1950s and 1960s (Barber *et al*, 2005a).

Hexachlorobenzene has a high octanol-water partition coefficient (log K_{ow} ~5.5) and high volatility. These properties are highly temperature dependent and together with its low degradability determine its fate in the environment. HCB is a multi-hop chemical, subject to long-range transport by what has been called the 'grasshopper effect' (Mackay and Wania, 1995). HCB is trapped in organic phases after atmospheric deposition, is not degraded and due to changes in temperature or sediment re-suspension volatilisation to the atmosphere takes place. Re-mobilisation of HCB has been observed, for example, in studies on the river Rhine and the estuary of the river Elbe. The global distillation process and long-range transport through the atmosphere have been given as a reason for its global distribution and the prevalence of HCB in remote polar regions (Barber *et al*, 2005a). Concentrations of HCB in the atmosphere are now

uniform, varying by less than an order of magnitude at background sites. This is evidence for its atmospheric persistence and long-range transport.

The high log K_{ow} of HCB determines its accumulation in lipid-rich tissues of biota. HCB concentrations have been measured in tissue of many different aquatic and terrestrial invertebrates, and measured aquatic bioconcentration factors (BCFs) of 3,000 to more than 35,000 have been reported (Barber *et al*, 2005a). Metabolism of HCB is usually low in invertebrates, but higher in mammals like gulls or seals. For substances with log K_{ow} values between 5 and 7 and low metabolisation rates, biomagnification via the food chain is expected to occur, and this has indeed been measured for HCB in different types of food chains (e.g. Antarctic and alpine lake food chains, Barber *et al*, 2005a).

Because of its bioaccumulation potential, persistence in the environment and long-range transport potential, HCB is part of the list of substances banned globally under the Stockholm Convention on persistent organic pollutants (POPs). Its use has been banned in nearly all countries and production has ceased, but HCB is still generated in small quantities as a by-product during chemical (mainly chlorinated solvents) and pesticide manufacture. Furthermore, thermal decomposition during uncontrolled waste incineration releases HCB into the environment. Levels of HCB in the environment have declined significantly since the production and use of HCB was banned. The decline was very rapid in the first years, but the rate of decrease has slowed markedly since the 1990s. The current estimated HCB emissions are a fraction of what is estimated to be required to maintain observed atmospheric concentrations (Barber *et al*, 2005a). However, the levels of atmospheric deposition have remained low but constant during the past years (US EPA, 2000). This can be partially explained by the 'grasshopper effect', but suggests the existence of secondary sources.

In 2002, Euro Chlor undertook a voluntary risk assessment for several chemicals related to the chlorine industry, including HCB (Euro Chlor, 2002b). The risk assessment was targeted on the North Sea marine environment, and followed the TGD of the European Existing Substances Regulation. Due to the high log K_{ow} of HCB, in addition to risk to aquatic organisms risk for sediment organisms and secondary poisoning had to be assessed. Potential risk was determined using two methodologies: The 'classic' TGD approach, comparing aquatic concentrations obtained from monitoring data to PNECs calculated using ecotoxicological information, and an alternative approach where concentrations in fish obtained from monitoring data were compared to a critical body burden (CBB) for fish calculated using a BCF factor. The CBB method is more realistic for a highly hydrophobic and persistent substance such as HCB, but also uncertain because less data are available on internal concentrations in exposed organisms, both on the exposure and the hazard side. The two approaches showed very low risk to aquatic and sediment organisms associated to the presence of HCB in the North Sea region. Furthermore, risk of secondary poisoning for sea birds and mammals was considered low. Only for some highly

sensitive organisms, like minks and ferrets, risk of reproductive failure due to intake of contaminated fish could be present, as found in other studies for the Canadian Great Lakes environment (Moore *et al*, 1997). In addition, a developmental risk associated to the presence of HCB could not be ruled out for fish-eating birds because no reliable data on bird reproduction and embryo development is available.

The same risk assessment approach was used to determine the risk of HCB secondary poisoning for an Arctic food chain, with polar bears, whales and gulls as top predators. Limited data available on the food web composition, food type and BMF partially hampered the assessment, but CBB risk quotients showed that top predators in Arctic food chains could be at risk (Peters *et al*, 2009).

The two available risk assessments performed for the North Sea and an Arctic food chain do not consider the re-mobilisation and volatilisation of hexachlorobenzene from soils and sediments. These processes should be taken into account in the PEC estimation. Monitoring data and environmental fate modelling approaches to perform the calculations are already available (Barber *et al*, 2005b). The available risk assessments show that even for PBT/POP substances it is possible to reasonably estimate the risk associated to their presence in the environment. The hexachlorobenzene case clearly illustrates the fact that for a highly persistent substance, lower emissions will lead to a lowered risk for exposed species after a certain period of time, but attention should be paid to highly sensitive species and ecosystems.

3.1.3.9 Hexachlorobutadiene

Euro Chlor produced a risk assessment report for hexachlorobutadiene (HCBD) in 2002 (Euro Chlor, 2002a). This assessment focuses solely on the aquatic environment in and around the North Sea. It uses both the PEC/PNEC risk assessment methodology and the critical body burden method to assess the risk to the environment that HCBD poses. HCBD has had many restrictions put upon its release. It is still released in small amounts as a by-product of some manufacturing processes. It is important to note that the aquatic compartment is not the only compartment that HCBD will partition to. In fact, Mackay modelling was used to predict that the lowest percentage of environmental HCBD would be partitioned to this compartment (0.2%), with 97.8% being partitioned to the atmosphere and 1.0% to both the soil and sediment compartments. The ECB TC NES sub-group has categorised this substance as POP, PBT and vPvB.

In order to determine the PEC of HCBD in water, the mean average and 90th-percentile environmental concentrations were calculated from a collection of monitoring data, including a database containing 10,000 measured HCBD concentrations from the rivers of six countries

around the North Sea. These were taken as typical and 'worst case' PEC_{marine} values (5 and 12 ng/L). For the marine sediments, using a similar approach, the typical and 'worst case' $PEC_{sediment}$ values were determined to be 1 and 4 µg/L, respectively.

There were insufficient data to compare the sensitivity of marine and freshwater organisms to HCBD. However, data from other chlorinated aliphatic compounds suggested that the sensitivities would be fairly similar. A chronic toxicity test on freshwater algae was deemed to be non-valid because the methodology and measurement endpoint were non-standard, however the result was deemed approximately equivalent to a NOEC and the result (>25 mg/L) was sufficient to indicate that algae were not the most sensitive species. Therefore a universal $PNEC_{aquatic}$ was calculated by applying an assessment factor of 50 to the only reliable NOEC value (0.0065 mg/L) for fish to produce $PNEC_{aquatic}$ = 0.13µg/L. The $PNEC_{sediment}$ was derived using the equilibrium partition method and the $PNEC_{aquatic}$, which produced a value of 24.4 µg/kg dry weight.

Calculation of the PEC/PNEC ratios for both typical and 'worst case' scenarios resulted in no values that were higher than 1 (the highest was the ratio for the 'worst case' in sediments, 0.16). This indicated that the levels of HCBD measured in both compartments were unlikely to represent a risk.

The critical body burden risk assessment was evaluated using bioconcentration and monitoring data. The CBB was obtained by multiplying the lowest NOEC (6.5 µg/L) with the BCF (17,000) to give a value of 111 mg/kg ww. This concentration was then compared to the HCBD concentration in samples of fish taken from around the UK. The maximum concentration found was 0.4 µg/L, which was well below the calculated CBB.

The risk of HCBD to fish-eating predators was also assessed to attempt to quantify the effects of HCBD biomagnification up the food chain. This was done by comparing the estimated daily intake (EDI) of HCBD through contaminated fish to a $PNEC_{oral/food}$ e.g. 0.2 mg/kg body weight/day (for rat). The mink EDI_{fish} (0.06 µg HCBD/kg body weight/day) was calculated by multiplying PEC_{fish} (estimated 0.4 µg HCBD/kg body weight) by feeding rate (FR) (0.15 kg/kg body weight) of predators. This value is several orders of magnitude below the $PNEC_{oral/food}$. This indicates that there is little risk to predatory species eating fish contaminated with HCBD.

Therefore, according to the risk assessment report produced by Euro Chlor, using refined methods for exposure assessment (based on monitoring data) and the critical body burden approach for the effects assessment, the ecological risk posed by the amount of HCBD which is released to the North Sea marine environment is acceptable. It may be necessary for a more general risk assessment to be performed which applies to the global environment, as the

conditions that exist in and around the North Sea are not representative of other marine regions. However, of the PEC/PNEC ratios calculated, none were remotely close to 1.

3.1.3.10 Lindane

Lindane (or γ-HCH) was a widely used agricultural pesticide. It is now listed as a "substance scheduled for restrictions on use" in Annex II of the 1998 Protocol on Persistent Organic Pollutants of the Convention on Long-Range Transboundary Air Pollution. It is banned in 52 countries and restricted or severely restricted in 33 countries, according to the North American Commission for Environmental Cooperation, 2006 (UNEP, 2007). In countries where it is still in use the only permitted agricultural application method by which it can now be used is through seed coatings, as it binds strongly to organic matter meaning that it will be unlikely to leach through soil to groundwater. This prevents lindane from being transported in aerosol form to bodies of water, as was the case when it was applied through sprays (Patterson, 2004).

According to the UNEP risk profile, lindane meets EU PBT criteria (UNEP, 2006).

The US Environmental Protection Agency conducted a risk assessment in their Reregistration Eligibility Decision (RED) for lindane (US EPA, 2002b). Ecotoxicity data were compared to estimated environmental concentrations (EECs), providing a screening-level assessment of the risk posed by lindane, at maximum application rates, for each crop upon which it was used. The treatment of seeds for lindane was the source of exposure to the affected species in the risk assessment. The EECs for terrestrial species were based on the quantity of lindane-treated seeds that a bird could consume, and assumed that the bird eats only lindane-treated seeds. EECs for aquatic species were produced using the Tier I-Generic Estimated Environmental Concentrations (GENEEC) model, which assumes that 100% of the substance used to coat the seeds will dissociate from them once planted and be able to migrate to surface water. These assumptions, as a result, provided a conservative estimate of the true exposure concentrations.

For terrestrial animals, both acute and chronic LOCs were exceeded for all seed treatment uses. For birds, the RQs ranged from 0.21 to 5.48 for acute risks and from 3.9 to 83.3 for chronic risks. However, research has shown that certain bird species will be repelled by seeds coated with lindane, suggesting that the RQs are yet more conservative. The human occupational risk assessment for lindane concluded that the current maximum application rate would be reduced by half, from 0.125 to 0.0558 lb ai/100 lb seed. Based on the evidence for a bird's feeding preference, and the measures already implemented based on the occupational risk, it was deemed that no further risk mitigation actions were required. For mammals RQs for acute exposure ranged from 0.24 to 2.1, and for chronic exposure, from 16 to 63. Mammals tend to hold territories, rather than living over large regions, so it was concluded that the risks posed to them

by lindane treated seeds could be confined to local populations. It was also stated that it was likely that mammals would be opposed to consuming lindane treated seeds, as birds are. For these reasons it was deemed that the risk mitigation actions already put in place were sufficient for the mammalian populations.

Acute RQs were also exceeded for aquatic species from both freshwater and marine / estuarine environments (the RQs for aquatic risk ranged from 0.04 to 12.2). No chronic LOCs were exceeded for aquatic species, although chronic risk to marine / estuarine fish could not be assessed due to a lack of toxicity data. In spite of this, in the knowledge that aquatic risks would be lower than the calculated RQs based on the conservative nature of the model used to predict the EECs, the agency concluded that it did not have risk concerns for aquatic species.

3.1.3.11 Musk xylene

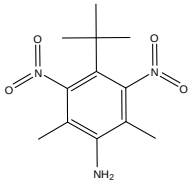
Musk xylene is one of a group of fragrance ingredients known as the nitro musks. The other major nitro musk is musk ketone. Musk xylene was used in a variety of fragranced products including fine fragrance, soaps, detergents and cosmetics.

An initial environmental risk assessment (RIVM, 1996) was carried out for musk xylene and musk ketone according to the EU TGD for Environmental Risk Assessment for New and Existing Substances. The initial risk assessment was based on information and results of studies provided by the fragrance industry as represented in the Netherlands by the Association of Fragrances and Food-Additives Producers (NEA), by the Research Institute for Fragrance Materials (RIFM) and international open literature. PEC/PNEC ratios, based on monitoring data, were < 1 except for the soil compartment. A subsequent EU Risk Assessment Report in 2005 (ECB, 2005) confirmed half-lives in marine water > 150 days (vP) and a subsequent addendum (ECB, 2008f) based on Japanese MITI data confirmed BCF values > 5,000 L/kg (vB). The International Fragrance Association banned the use of musk xylene following its Code of Practice for Environmental Standards based on its vPvB (hazard) characteristics in 2009. The European Commission has recently announced its decision to ban musk xylene under REACH, bringing EU regulations in line with the global IFRA standards.

Analytical methods are available for determination of musk xylene (MX) and musk ketone (MK) together with their transformation products 4-amino-MX, 2-amino-MX, and 2-amino-MK in aquatic environmental samples at a ng/L level (Gatermann *et al*, 1998). Transformation of nitro musks to the corresponding amines was studied in Hamburg STP samples and in the river Elbe. Much higher concentrations (4-40 x) of the amino derivatives of MX (2-amino-MX and 4-amino-MX) and MK (2-amino-MK) were found in effluent samples compared to their parent compounds. The simultaneous decrease in the nitro musk concentrations and the increase in the

amino derivatives suggest that, besides adsorption on the sludge, this transformation pathway plays an important role in reducing environmental exposure. Future monitoring programmes as well as risk assessments of nitro musks in aquatic ecosystems should include the transformation products such as the amino derivatives.

A limited risk assessment was carried out on the major metabolites of musk xylene and consequently there are concerns about the need for further confirmatory data. The metabolite structure of the metabolite, 4-amino MX is shown below:



The aquatic risk assessment of these metabolites was presented in the ECB (2005) report. The TC NES noted that "comparing the PNEC of 0.4 μg/l with the measured value of 2 ng/l in the Elbe river (Gatermann *et al*, 1995; 1998), indicates that there seems to be no direct reason for concern for the 4-aminometabolite."

Based on the existing hazard and risk assessments, several possible recommendations have been made and could be considered:

- Persistence: Investigate the role of photolysis in overall persistence of musk xylene.
- Toxicity: Consider the outcome of additional studies recommended in the addendum (FELs, Fish early life stage).
- Metabolites: Investigate the risk assessment of these materials in other media either through models or laboratory studies.

While the additional studies would likely have added clarity and reduced uncertainty to the risk assessment of musk xylene, it would have been unlikely that they would have changed the outcome of the hazard assessment.

3.2 Metabolites / degradation products: Implications for (PBT) risk assessment

Within the risk assessment of all chemicals, regardless of PBT, vPvB or POP categorisation, an understanding of the formation of stable metabolites and transformation products is a critical component in order to fully assess environmental risks. These metabolites may persist in the environment, have the potential to bioaccumulate and cause toxicity at low concentrations. They

may even present greater environmental risk than the parent material. In principle, metabolism is a process that aims at making chemicals more polar and easy to excrete, therefore reducing (in general) their bioaccumulation potential and toxic potency. However, some substances may be transformed to metabolites or transformation products through environmental processes that will present a new source of risk, which must be quantified.

For example, some PBT/POPs have been identified due to the environmental properties of their metabolite or transformation product, sometimes formed through slow environmental transformation. Examples include aldrin which transforms to dieldrin which is much more persistent than the parent material (Boethling *et al*, 2009) and DDT which partially degrades to form the recalcitrant metabolite DDE. For DDT/DDE, a US monitoring programme, the National Health and Nutrition Examination Survey (NHANES), found that the metabolite of DDE is detected in almost all participants, even though the use of DDT was banned in the United States in 1972 (Swackhamer *et al*, 2009). This even applies to those participants born long after the ban, highlighting the recalcitrant nature of DDE and the importance of considering metabolites and transformation products within risk assessment of high priority chemicals such as those categorised as PBT/vPvB.

Due to the nature of most metabolic processes, the formation of such recalcitrant metabolites is not common. However, when it does occur the environmental risk of these chemicals must be assessed in order to fully understand the environmental consequences of the exposure to the parent material. Such a risk assessment can be conducted in principally the same way as the risk assessment for the parent material, as discussed previously within this report.

On a screening level, QSARs may be used to predict the identities of degradation products and estimate their PBT properties. Theoretical biodegradation properties can be derived using the University of Minnesota Pathway Prediction System (Fenner *et al*, 2008) and CATABOL (Jaworska *et al*, 2002; Dimitrov *et al*, 2002). For more detailed and confident determinations, *in vitro* or *in vivo* studies using sophisticated analytical methods, such as modern mass spectrometric techniques, are useful.

QSARs (e.g EPISuite [US EPA, 2009]) may also be useful, within a screening level assessment, to predict the environmental properties of the expected metabolites. In addition, some understanding as to the likely degree of exposure of the metabolites, based on their intrinsic properties (predicted or measured), can be used to build a screening level risk assessment in order to understand the need for further refinement. For example, an understanding in what compartment the transformation process took place (i.e. the new source); and in what proportions it is produced, given the multiple likely degradation pathways, which will depend on the compartment and conditions. The generation of toxicity data may present complications as the metabolite is unlikely to be commercially available and, if it was formed through a non-abiotic

process, it may be difficult to synthesise for testing. The use of modelling no doubt forms a crucial part of the risk assessment of these substances.

For the substances reviewed in this chapter, a brief review of the major, or likely, metabolites can be found in Appendix B.

3.3 Summary and conclusions to risk assessment methodologies used in the examples

Some of the problems associated with inadequacies in the estimation of the risks of PBT/vPvB and POP substances stem from the difficulty in accurately assessing and understanding the impact of the Persistent, Bioaccumulative and Toxic properties by which they have initially been discriminated from other substances. The uncertainty and potential for error when conducting testing for properties related to PBT criteria increases for substances possessing P, B and T properties due to the fact that these characteristics are often outside the scope of conventional test methods.

In many cases the risk assessment methods used in risk assessments of PBT/vPvB/POP substances followed the traditional PEC/PNEC methodology. In most cases they have employed the use of standard modelling techniques to predict environmental exposure concentrations (PECs) from emission data and the physico-chemical and fate properties of the substance, and compared these to predicted no effect concentrations (PNECs) which are derived through limited standard toxicity testing data and application of assessment factors.

These case studies also demonstrated that many aspects of uncertainty still need to be addressed. They include: A lack of transparency and consistency in some aspects of regulatory decision-making; making decisions in the absence of sufficient exposure and effects data; placing too much reliance on model-based data and screening level data to support definitive assessments; relying on predicted rather than measured environmental concentrations; a focus on applying the precautionary principal rather than taking due consideration of environmental relevance through conducting a robust exposure assessment and targeted secondary uptake and appropriate chronic effects data; extrapolating long environmental half-lives from short laboratory-based fate studies (e.g. SCCP have a half-life of 1630 days in freshwater that is derived from a 100-day study); and the applicability or relevance of certain tests and endpoints (e.g. log K_{ow} as a trigger for ionisable chemicals and testing at or above water solubility levels to determine degradation rate constants). Some of the risk assessments for the substances described above have deviated from standard methodology in order to reduce the uncertainty of the conventional risk assessment methods. The main refinements that were used are:

- Exposure assessment:

- Overall persistence (P_{ov}) of HBCD has been investigated using the RAIDAR model and The Tool.
- Biomonitoring data have been collected to refine the exposure assessment (HBCD).
- An advanced fate model was used that allowed the prediction of spatio-temporal variation in concentrations (TBTO and HBCD).
- For endosulfan, a probabilistic assessment of reported application data was made.

- Hazard assessment:
  - For HCB, HCBD and HBCD, the CBB approach was used to refine the PNEC estimation.
  - Tolerable Daily Intakes were calculated based on available data (HBCD and HCBD).

The next chapter of this report will focus on the refinement options that could be applied in the risk assessment of PBT/vPvB substances. Some of them have already been applied in the reviewed risk assessments for PBTs and POPs, others are under development. An attempt will be made to highlight their advantages and the challenges associated to their application. In a previous ECETOC Technical Report on "Risk Assessment of PBT Chemicals" (ECETOC, 2005a) a methodology was proposed which attempts to address the special considerations required for the accurate risk assessment of PBTs. This methodology involves starting the assessment from a 'higher tier' compared to normal methodologies by considering, in addition to the standard risk assessment data, all data now available as a result of testing to inform the PBT categorisation. This aims to reduce the increased inherent uncertainties involved when attempting to characterise exposure and risks of these substances. It also recommends that, due to the special fate properties of these substances, exposure estimations are extended to areas that are remote from emission sources (PEC refinement) and to exposure through secondary poisoning (PEC_{oral} consideration) to account for food chain bioaccumulation. It also suggests that potential effects of these substances are assessed in greater detail (PNEC refinement), by considering higher tier effects data, such as chronic data, critical body burden measurements and identifying the true mode of action. Conventional risk assessment strategy as applied to non-PBTs will not necessarily take into account all of these considerations and therefore will not necessarily come to a conclusion about risk that is both comprehensive and protective for PBT/vPvB substances.

The proposed methodology describes a refinement process consisting of three phases of increasing specificity: The evaluative phase, investigative phase and confirmatory phase. These are presented in schematics for each possible risk assessment element (PEC, PEC_{oral} and PNEC) which, whilst not being prescriptive, gives an indication of how refinements to the risk assessments may be targeted to ensure accurate characterisations of risk. For example, refinements of PEC have been broken down into considerations of emissions data, physico-chemical and partitioning data, degradation data and modelling and monitoring.

Each consideration is a separate element of PEC determination, which carries its own uncertainties and may be refined separately to be incorporated into the overall risk assessment. Also, each element is described in terms of what sort of data it may be characterised by for each of the three possible refinement 'levels'.

Chapter 4 of the current report indicates particular refinements following new methodological developments which (if utilised) could all be accommodated by this refined risk assessment strategy.

4. REFINEMENT OPTIONS

Before a risk assessment is conducted on a chemical categorised as PBT or vPvB it is assumed that the categorisation step has been completed and any issues resolved. Through this step a significant amount of information would be available on the chemical fate and effects. The refinement options given within this Chapter are proposed as ways of providing additional information necessary to conduct higher tier risk assessment than could be conducted with the data necessary for PBT/vPvB categorisation. Using these approaches should reduce uncertainty and contribute to a more environmentally realistic risk assessment.

The options below are intended as developments to the approaches given in the previous ECETOC report on 'Risk Assessment of PBT Chemicals' (ECETOC, 2005a). Advancements in scientific approaches are considered as well as experiences gained in the risk-based evaluation of high priority chemicals such as those categorised as POP, as reviewed in Chapter 3.

PBT substances are difficult to characterise using standard test methods. These deficiencies in standard testing methodology imply that more refined, higher tier testing and correct interpretation of results will be required in order to determine if a chemical is a PBT/vPvB. From an exposure perspective, the high persistence and bioaccumulation potential of PBTs, and in the case of POPs, the long-range transport potential, mean that it is difficult to ascertain how far reaching the effects of these substances may be on the open environment. A standard ready biodegradability test will only confidently indicate a positive or negative result for ready biodegradability, but will in many cases not measure the true biodegradation potential of a substance (rate or pathway). There can also be a large degree of variation in the observed degradation rates in these tests. Therefore, a definitive assessment of the persistence of a substance cannot confidently be assessed from these methods unless a sufficient body of degradation data exists to assign some confidence to the measured rates. Previous work by ECETOC (2009) has reviewed the half-lives assigned to readily biodegradable chemicals and has shown that they over-estimate the persistence of chemicals in marine and freshwaters. However, insufficient data existed to review the basis of the half-lives (150 days) assigned to non-readily biodegradable (e.g. inherently biodegradable) chemicals.

Current screening criteria for bioaccumulation potential only consider bioconcentration factor (BCF) as a requirement to determine this parameter. However, BCF is only an indicator of accumulation of a substance in an aquatic environment, through direct uptake from the medium. Therefore, not only is an uptake of substance through the atmospheric environment or the diet not currently regarded, but there are also no set criteria for establishing whether or not a substance can biomagnify through the food chain to reach significant quantities in higher organisms. This is a major concern for PBT/vPvB/POP substances, for which a significant research effort is on-going; yet no standard techniques are defined to incorporate these methodologies into an

assessment approach at present. In some of the examples reviewed in the previous chapter (e.g. HBCD) existing data on biomagnification have been considered in certain food chains.

Both monitoring data and environmental modelling are crucial tools in exposure assessments. Some of the risk assessments reviewed in the previous chapter utilise them either alone or in combination. The latter would be the preferred method as the strengths of one may complement the other one and *vice versa*. It is the nature of environmental models that they are continuously under development, and the understanding and quantification of the dynamic environment is a highly complex task. Monitoring data are therefore essential in validating model predictions, reducing uncertainty and adding to the confidence in their predictions. Modelling data are equally important in expanding on data in monitoring databases to build up a larger picture of the environmental distribution of substances, as it is not practically possible to take measurements from every location.

With regard to the effects assessment there are several reasons why the standard techniques of deriving PNECs by applying assessment factors to routine toxicity test results are to be used with caution for PBT substances. Toxicity screening tests conducted over acute timescales will often terminate before significant quantities of the substance have been taken up by the test organism that could lead to a substance-related chronic effect. In these cases the true toxic nature of a substance may be misinterpreted. On the other hand, if toxicant modes of toxic action are specifically targeting certain organs or pathways they will add uncertainty to the process, since there can be a large variation in dose-response relationships between species and endpoints. Therefore it is important to establish whether this is the case, by building a larger suite of test data, assessing a range of organisms, in order to determine the most sensitive species. Knowledge of the mechanism of action on target species for specifically acting chemicals can help ensure that the most sensitive species are included within the testing programme. The bioaccumulation potential of PBTs can imply that toxicity testing may not result in the true extent of toxic effect being observed. This may be as a result of internal toxicity thresholds having not been exceeded as sufficient time has not been allowed for the uptake / removal processes to reach steady state. PBT/vPvB suspected potential to biomagnify up the food chain also poses the problem that testing the toxicity to receptors of concern (i.e. top predators) carries with it many restrictions. However, data from mammalian toxicology can be successfully used under certain conditions. Toxicokinetic data and modelling results can be used to reduce the uncertainty in PNEC of NOAEL derivations, define time to steady state and relate this to the life time of the species of concern. Some of the examples reviewed above have used the critical body burden approach, some modelled steady state concentrations in different species and some used refined mode of action data for different species to refine the effects assessment.

The previous paragraphs show that new experimental and theoretical frameworks have been developed in the past decades to aid the evaluation of the PBT properties of chemicals. Reports

and scientific articles exist which detail these developments, their strengths and the pitfalls associated to their application (see ECETOC, 2005a; 2005b; 2007a; IEAM, 2009 for summary publications). In this chapter, an attempt to lay out the state-of-the-art methodologies applied in the evaluation of fate and effect properties of chemicals has been made, using the available risk assessment examples as case studies when possible. The main improvements are related to enhancements in fate / effect modelling and (bio)monitoring techniques. The improvements described below are essential for conducting risk assessments for any kind of chemical, but are especially important for PBT chemicals, which call for the use of tailored methodologies due to the uncertainties around their fate and effects.

4.1 Chemical space mapping as a tool to help guide risk assessment refinement strategies

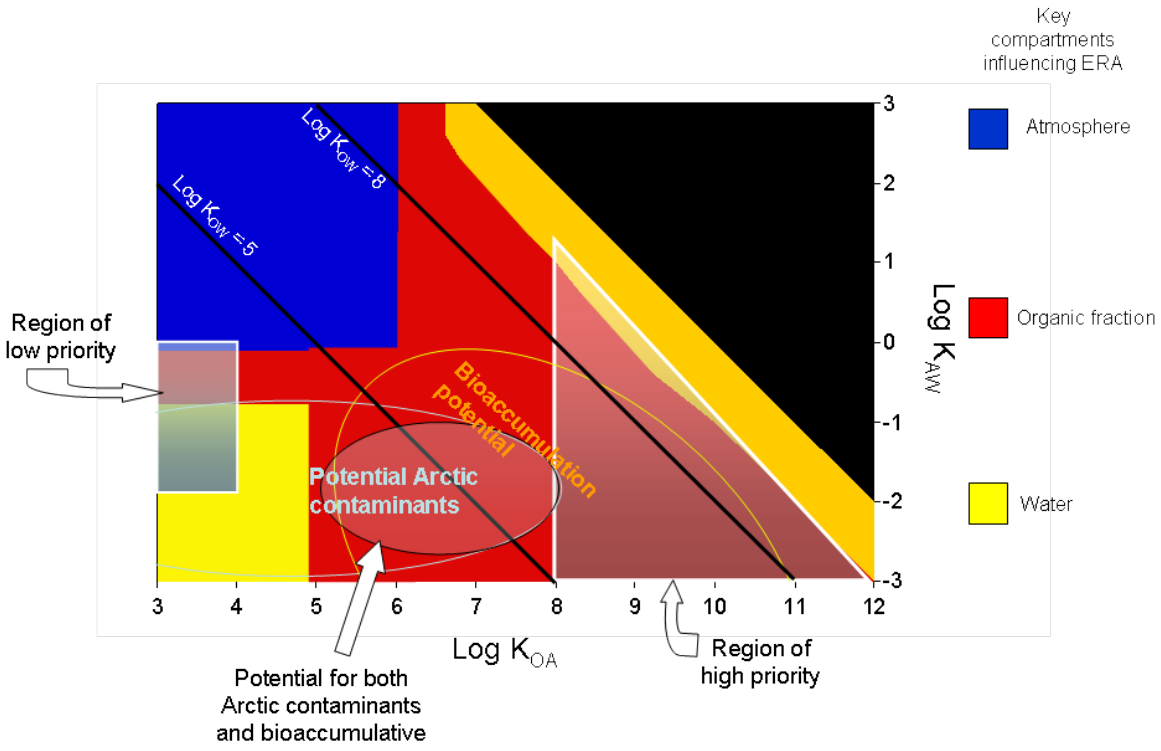
Chemical space mapping is an approach that uses equilibrium partitioning property data (K_{oa}, K_{aw}, K_{ow}) to predict the fate of a chemical. Such chemical space mapping has been used in a simple evaluation of the key half-life influencing the persistence of a chemical (Gouin *et al*, 2000); to predict how chemicals will be transported, particularly to remote environments (Wania and Dugani, 2003; Wania, 2006); in linking persistence, long-range transport and potential for bioaccumulation (Czub *et al*, 2008); and to inform priority setting in regulatory decision making for substances of high concern (Gouin, 2010).

These applications of chemical space mapping suggest that the approach could be used to help direct a testing strategy for substances categorised as PBT or vPvB. For example, chemical space mapping may help with questions such as: Should emphasis be placed on improving estimates of degradation in air, water, soil or sediment? Should a testing programme be implemented to assess the presence of a chemical substance in remote environment? Is there a need to better assess the bioaccumulation and effects of substances using high tier approaches such as TMF and CBB? Figure 1 builds on the approaches used by Gouin (2010) to illustrate how chemical space mapping can be used to inform the refinement strategies of chemicals categorised as PBT or vPvB.

Figure 1 shows how the K_{oa}, K_{aw} and K_{ow} partitioning coefficients can predict the primary compartments of concern. For example, a substance with a log $K_{oa} < 6$ and log $K_{aw} > 0$ will be expected to partition predominantly to the air compartment. Therefore, within a persistence assessment of such a chemical the atmospheric half-life would be a key factor. Similarly, substances with log $K_{ow} > 4$ with log $K_{aw} < 0$ and log K_{oa} between 5 and 11 are expected to show a potential to bioaccumulate. As a consequence, the risk assessment of these substances should consider a high tier food chain assessment that may include aspects such as TMF, CBB as well as biomonitoring in the environment across trophic levels. When this bioaccumulation potential is

linked with a potential to transport to remote areas (i.e. log K_{oa} between 5 and 8 and log K_{aw} < -1) then food chain modelling and biomonitoring should consider Arctic food webs.

Figure 1: Use of chemical space mapping to inform chemical risk assessment refinement strategies



The information that can be gained from chemical space mapping approaches can be combined with detailed knowledge of the use scenarios for the substance in question. For example, is the substance used in a wide, dispersive manner or within a small number of sites or operations? Using this information within appropriate environmental fate models, can the fate within local or regional compartments be predicted? Can the estimated PECs be validated through monitoring data? Is an appropriate monitoring programme that takes account of spatio and temporal variations necessary? What organisms can be expected to be exposed to the chemical (e.g. freshwater, marine or estuarine sediment organisms)? Using information (modelled and/or measured) in relevant environmental compartments, can a body burden be predicted and/or measured within the most sensitive trophic level? Can body burdens then be predicted and/or measured within higher trophic levels? Can adverse effects be expected within each of these trophic levels, either acutely or chronically? Is there environmental evidence of these body burdens and effects? And so on.

4.2 Exposure

Though bioaccumulation of a chemical is the result of absorption, distribution, metabolism and excretion (ADME) processes, most models neglect the contribution of metabolism as a clearance mechanism, potentially leading to inaccurate estimates of bioaccumulation potential. In addition, no currently available models are capable of accurately estimating potential rates of metabolism. Though the *in vivo* OECD 305 fish bioconcentration factor (BCF) test (OECD, 1996) accounts for metabolism, it requires large numbers of test organisms and is time-and cost-intensive. There is a need to close this gap between *in silico* and *in vivo* approaches with an efficient *in vitro* testing strategy that includes not only an assessment of passive uptake but also biotransformation and proper scaling from test tube to whole animal.

Incorporating xenobiotic metabolism by Phase I and II metabolising enzymes in fish is critical to improve bioaccumulation estimates (Smítková *et al*, 2005; de Wolf *et al*, 2007). These *in vitro* metabolism data can be incorporated into BCF prediction models and extrapolate *in vitro* test results to whole body biotransformation rates (k_{met}) to refine BCF model predictions (Cowan-Ellsberry *et al*, 2008).

One crucial issue when assessing the environmental risks of PBT/vPvB substances is the difficulty of accurately predicting environmental concentrations to be compared with estimated effect concentrations. In many cases environmental concentrations in the example risk assessments in this report were estimated, based on information on the substance uses, by basic screening models such as those incorporated into EUSES. Whilst, in most cases, the estimations of such models are considered to be conservative, the persistent and bioaccumulative nature of PBT substances will most certainly cause inaccuracies in predictions of environmental concentrations. For example, the EUSES model will calculate an environmental distribution of a substance on two spatial scales: The local scale and the regional scale. The local scale implies the concentrations of a substance expected within the immediate vicinity of a point source of emissions. The regional scale represents an area away from the point source where some of the substance may diffuse to via transport through the various compartments. It is considered to be constant throughout the area and does not have a dependency on distance from the source, nor does it differentiate based on unique environmental conditions. However, the very nature of PBTs implies that they are capable of persisting for greater lengths of time, may build up in significant quantities in biota, and may be transported over distances remote from source of emission. This sort of occurrence is not considered in the basic modelling of typical risk assessment programs and hence important routes of exposure and risk may be omitted from assessments conducted using these standard methodologies. Temporal considerations are also excluded from these modelling techniques – predicted environmental concentrations are calculated purely based on the present tonnages input at the time of assessment. Therefore an increase of concentration of substances over long periods of time, and a prediction of the time for

environmental concentrations to reduce after the cessation of releases, are not considered. It is therefore important to employ more refined methods of estimating environmental concentrations, either through using more detailed modelling techniques or validating estimated environmental concentrations using representative monitoring data.

In this section all recently developed refined methodologies that influence the exposure assessment will be reviewed. This includes developments on the assessment of persistence and food chain bioaccumulation as well as the determination of exposure concentrations in environmental compartments or target organisms including humans.

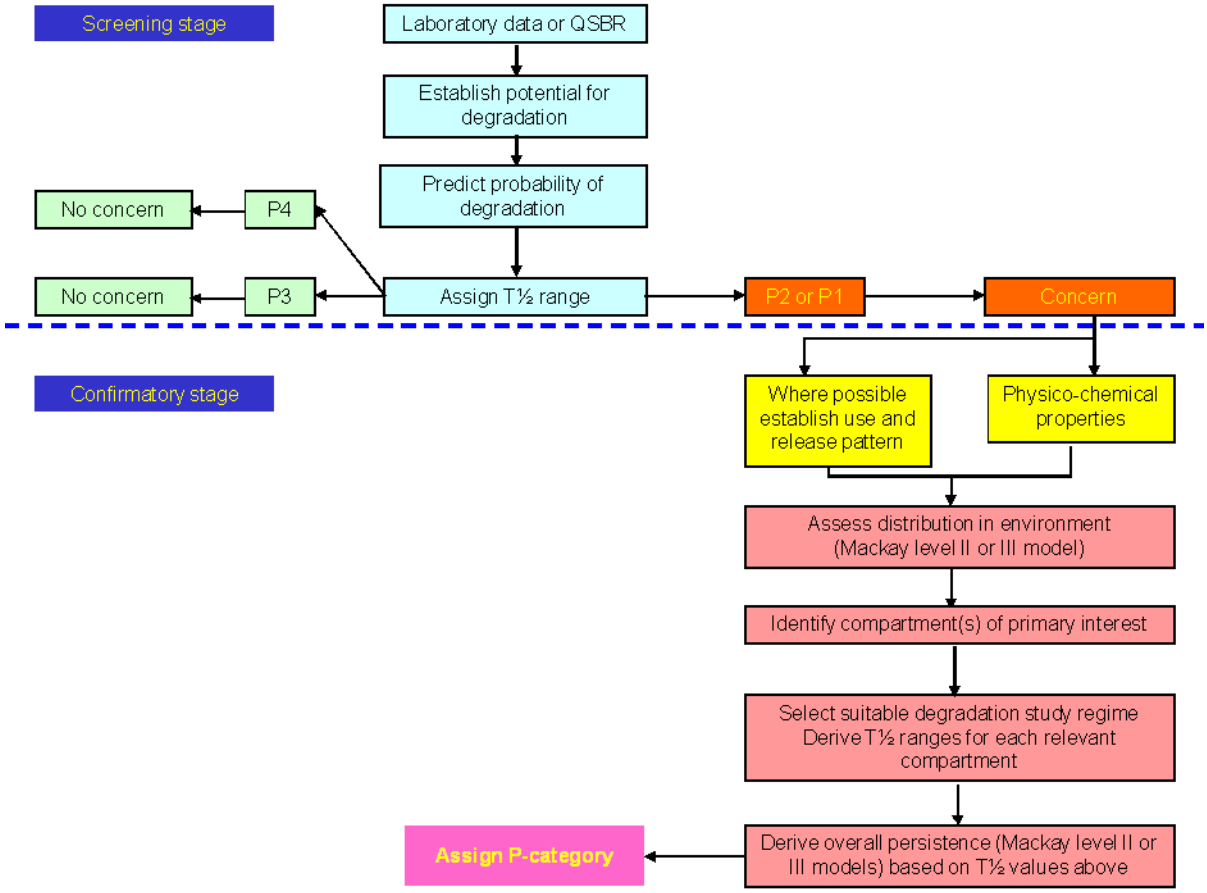
4.2.1 Persistence assessment

4.2.1.1 Single media persistence

The persistence of chemicals in individual environmental media has been addressed extensively in recent years and various approaches have been advocated to assign individual half-lives for persistence in individual media at a screening and confirmatory level (Boethling *et al*, 2009; ECETOC, 2003; 2005a,b; 2007a; 2009; STEP, 2004). ECETOC (2009) and Boethling *et al* (2009) have summarised many of the approaches that have been advocated to assess single media persistence. In addition to providing integrated guidance for persistence assessment, many of these activities also identified the need for better screening tests to provide a more robust approach to persistence assessment and prioritisation (ECETOC, 2003; 2007a; Boethling *et al*, 2009).

Boethling *et al* (2009) identified a number of common themes that are recommended in these various approaches. The most prominent is the recommendation of a tiered approach to single media persistence assessment, in which a chemical is first examined at the screening level, followed by further scrutiny in a confirmatory phase, but only if certain conditions are met. Other common themes from the existing persistence guidance identified by Boethling *et al* (2009) included maximising the use of existing standard and non-standard data, using multimedia models to identify and prioritise the most important environmental compartments, and using this information to help determine the types of persistence data that would be most useful (thus testing, if testing is needed). Figure 2 illustrates the two-tiered approach advocated by ECETOC (2003) that is aimed at maximising the use of all test data in the screening phase to attempt to make a conclusion on environmental persistence before targeting a more detailed environmental assessment in the confirmatory phase.

Figure 2: ECETOC persistence task force approach for evaluating environmental persistence



Boethling *et al* (2009) identified that multimedia modelling had a significant, but perhaps hitherto under-appreciated (and less frequently applied), role as a higher-tier tool in POP and PBT assessment. In addition to identifying and prioritising environmental compartments in which to measure degradation, Boethling *et al* (2009) recommended using multimedia models to evaluate the relative importance of persistence data from two or more environmental compartments. This approach would help determine where the weight of experimental evidence lies. Boethling *et al* (2009) stated that, provided the data meet quality expectations, half-lives for those compartments predicted to contain the highest mass fractions should be given the greatest weight in assessing whether a given chemical meets or does not meet the applicable P criteria, because the chemical is predicted to partition there (or is emitted directly), and degradation is not rapid enough (in relative terms) to result in low concentrations.

A significant amount of the guidance and recommendations made by ECETOC (2003; 2005a) and STEP (2004) on how to approach the assessment of environment persistence were captured in the technical guidance provided by REACH (ECHA, 2007). The REACH technical guidance

for degradation and persistence (ECHA, 2008a,b) also identified some new test-based approaches for assessing persistence that recognised the inherent problems associated with the current biodegradation screening tests. Many of these inherent problems had been described by ECETOC (2003; 2007a) and STEP (2004). As a result REACH (ECHA, 2008a) identified two new 'tiers' of tests; these were the modified ready biodegradation tests and the enhanced biodegradation screening tests.

The modified ready biodegradation tests allowed two simple modifications to the ready biodegradation to be made (ECHA, 2008a). These modifications did not seem to alter the nature of the microbiology of the test system; they addressed the manner in which the test chemical was presented. The first modification allowed the use of low test chemical concentrations to negate any toxicity that resulted from testing in the 2-100 mg/l range. The second modification recognised mass-transfer problems with conducting studies in the 2-100 mg/l range and formalised the use of the ISO guidance for enhancing the bioavailability of poorly water-soluble chemical substances (ISO, 1995). As no change was made to the nature of the microbial inoculum, chemicals that passed the criteria for these studies could be assumed to be readily biodegradable.

The enhanced biodegradation screening tests were identified to enable a more robust prioritisation based on persistence and lack of ready biodegradability. REACH (ECHA, 2008a) identified four possible enhancements that included (i) increased test durations; (ii) working with larger test volumes (to increase the total number of microbial cells in the test vessel); (iii) testing at increased cell densities; and (iv) using test systems that allowed for low-level laboratory pre-exposure or adaptation using environmental waters. Chemicals that degraded rapidly in these studies could be considered non-persistent but not readily biodegradable.

Whilst many of these activities have focused on developed tiered or intelligent approaches to single-media persistence assessment, ECETOC (2007a) and Boethling *et al* (2009) recognised that there was still a need for guidance on a variety of technical issues. These included the interpretation or evaluation of higher tier data with respect to:

1. Identification and assessment of transformation products (discussed earlier);
2. determining half-lives from dissipation kinetics;
3. identification of bound residues and whether they should be considered as part of the removed or degraded fraction;
4. adjusting degradation half-lives for environmental variables, such as temperature and pH. ECETOC have started some work on the issues associated with identification and risk assessment of bound residue through a workshop with regulatory and academic parties (ECETOC, 2010a).

Escher and Fenner (2011) have recently proposed risk-based approaches to address the issue of transformation products.

In many cases assessment of single media half-lives will be adequate to conclude on the persistence of chemicals in the environment to support a PBT assessment. It is also known that single media half-lives tend to provide conservative assessments of environmental persistence (Scheringer *et al*, 2009).

For certain chemicals that have multiple environmental discharges and undergo multi-media transport, including many of the POP-like chemicals, single media persistency assessments may not reflect the overall fate and behaviour of the chemical in the environment. These types of chemicals need a more holistic assessment of overall persistence.

4.2.1.2 Overall persistence

The concepts of overall persistence (P_{ov}) and long-range transport potential (LRTP) of chemicals in the environment were developed in the 1980s and 1990s (Mackay and Paterson, 1982; Klöpffer, 1994; Scheringer, 1996; Bennett *et al*, 1998, Beyer *et al*, 2000). Persistence of chemicals is commonly used to describe the ability of chemicals to remain present in the environment for a long time; whereas transport potential is used to describe the ability of chemicals to move through the environment over long distances (Scheringer *et al*, 2009).

Overall persistence is not mentioned in any of the legislation defining criteria for identifying POPs or PBT chemicals (Scheringer *et al*, 2006; 2009). The half-lives described in these legislative documents are restricted to single media determinants of persistence. However for the identification of POPs and some PBT chemicals, P_{ov} and LRTP can be important indicators for chemicals with multiple entries into the environment or where significant multimedia transport occurs.

According to Annex D of the Stockholm Convention, a chemical is classified as having potential for long-range transport if:

- It is measured in remote locations.
- Monitoring data show that long-range transport (LRT) has occurred.
- Environmental fate properties and/or model results indicate LRTP.
- The chemical has a half-life in air > 2 days.

Scheringer *et al* (2009) propose that a P_{ov} threshold is established that aims to deal with the total burden a chemical has in a global multimedia environment. A value for P_{ov} of approximately

90 days was identified by Scheringer *et al* (2009) as for most agrochemicals it would result in a minimum carry-over from one year's application to the next one.

Many single media approaches to persistence try to take account of multi-media persistence by making a simplistic assumption that chemicals undergoing long-range atmospheric transport have a half-life > 2 days in air, and undergo subsequent atmospheric deposition that a single media evaluation of persistence in the marine environment will be protective of the wider environment (STEP, 2004; ECHA, 2007).

A number of different metrics have been proposed and used to measure P_{ov}. A common theme is that they:

1. All aim to measure elimination of the substance by transformation, recognising that transport from one place or medium to another is not regarded as disappearance from the overall environment.
2. All focus on the overall environment, recognising the differences in reactivity of chemicals in different environmental media. Following earlier suggestions by Mackay and others (Mackay and Paterson 1982; Webster *et al*, 1998), the SETAC Fairmont workshop (van de Meent *et al*, 2000) identifies three options for measuring P_{ov}:
   - Elimination time: The time necessary for disappearance of a substance from the overall environment (air, water, sediment, soil, biota) to a certain extent (e.g. 10%), after cessation of all emissions.
   - Fraction remaining: The mass of substance that is still present in the overall environment at a certain time after emissions have ceased (e.g. 30 years) as a fraction of the mass present at the time when emission stopped.
   - Steady-state residence time: The time describing the 'turn-over time' of the chemical in a flow-through system, typically a level II or level III multimedia model with constant emission and loss processes balancing this emission. This residence time is calculated as the ratio of the mass present in the system divided by the emission mass flux.

According to Scheringer *et al* (2009) the most common metric for P_{ov} is the steady state residence time. This residence time will depend upon the distribution of the chemical between environmental media and the degradation and removal process that occur within these media.

Advantages of P_{ov}

The use of single media half-lives as criteria to decide on chemical persistence in the environment is relatively straightforward. However, some additional aspects can be important in the

evaluation of POPs, and some PBTs are not covered by the single media approaches advocated by REACH (ECHA, 2008a). These include:

- It enables chemical comparisons to be made against a single scale. To enable this, the different half-life criteria need to be integrated into a single metric, i.e. P_{ov}. This may help avoid problems caused by the single media approach to chemicals by having a metric that operates irrespective of the particular combination of media-specific half-live values.
- P_{ov} results can be linked to field data to determine the long-term decline and total burden of a chemical in the environment.
- P_{ov} can be related to environment burden or exposure in a more holistic sense that considers exposure from multiple sources.
- Single media half-lives can be precautionary and provide a 'worst-case' estimation of persistence as half-lives can be exceeded for environmental compartments that are not representative of those that will be exposed.

In addition, an assessment of P_{ov} and LRTP at a screening level using the OECD P_{ov} and LRTP screening tools can provide additional key information to help conclude on PBT and POP assessment. These screening tools allow chemicals to be benchmarked against well-studied chemicals including acknowledged POPs. Where uncertainties remain focused, data gathering may help improve the P_{ov} assessment process.

Common limitations of overall persistence evaluations

In order to achieve a reliable evaluation of P_{ov} and LRTP, good quality physical and environmental property data are required and in many cases these data can be inconsistent and incomplete (Boethling *et al*, 2009; Scheringer *et al*, 2009). The uncertainty in degradation half-lives and partitioning coefficients needs to be characterised and taken into account early within a chemicals persistence assessment. Multimedia models require at least two partitioning coefficients and three or four degradation rate constants. The most common pure substance properties and partitioning properties required include:

- Melting point (MP).
- Boiling point (BP).
- Vapour Pressure (VP).
- Water Solubility (WSol).
- Octanol / water partition coefficient (K_{ow} or P).
- Octanol / air partition coefficient (K_{oa}).
- Air / water partition coefficient (Henry's Law Constant).
- Soil / organic carbon partition coefficient (K_{oc}).

- Fraction of substance sorbed to atmospheric particulates (phi).
- Dissociation constant (pKa or pKb).

Great care is needed when physico-chemical property data are compiled for PBT and POP assessment and any outliers should be excluded from the dataset (Scheringer *et al*, 2009).

An absence of single media half-life data also restricts P_{ov} assessments. For chemicals where no half-life exists, new studies should be conducted and interpreted to generate the most appropriate single media half-life data according to the guidance provided by ECETOC (2003), REACH (ECHA, 2007) and Boethling *et al* (2009). Alternatively, predictive software such as BIOWIN and AOPWIN could be used; however these are likely to provide conservative assessments. Where degradation data do exist the degradation half-lives typically have higher levels of uncertainty than the physico-chemical property data (Fenner *et al*, 2003; ECETOC, 2009). In many cases these data will describe partitioning and not degradation as dissipation and no degradation times are reported. It is also difficult to assess and interpret many of the higher tiered biodegradation studies designed to generate DT_{50} values or half-lives as they have no validity criteria or positive reference controls.

For many chemicals, the absence of quality data, whether emission, degradation or partitioning data, is restricting the application and use of overall persistence assessments.

4.2.2 Modelling Action

Modelling of environmental exposure has a key role to play within any risk assessment approach. The costs associated with sufficiently robust monitoring programmes can be significant where chemical concentrations are measured over a spatial and temporal scale within relevant compartments. Modelling science continues to advance and the use of appropriate models with quality input data is key to targeting monitoring programmes. These monitoring programmes can then, in turn, support the further development of more predictive models.

Modelling helps to target monitoring, and monitoring can be helpful to validate and corroborate the models. There are a number of strategies for using models when evaluating the exposure of PBTs and POPs (Cowan-Ellsberry *et al*, 2009).

The aim of the exposure assessment is to establish the link between release of a chemical into the environment and its exposure to receptors of concern. Hence, if exposure is to be estimated using modelling, two models are generally needed: A fate model (i.e. the concentration in environmental compartments) and a bioaccumulation model (i.e. the internal concentration). With POPs there is the additional challenge of assessing the long-range transport to remote regions (this can be

integrated into the fate model, or calculated separately). There are currently no widely available modelling tools that incorporate these three aspects, although the fraction of an emission travelling to a remote region (e.g. the Arctic) can be estimated using one of the metrics from the literature. A fate and bioaccumulation model can then be run once this fraction has been established.

There are several ways in which a model can be employed to aid in the evaluation of POPs:

1. Groundtruthing.
2. Confronting uncertainty.
3. Benchmarking.
4. Predicting.
5. Forecasting.
6. Scenario testing.

1. Groundtruthing by comparing model results and monitoring data

Modelled results can lend credibility to existing monitoring data, physico-chemical properties and emissions estimates. This also serves to aid the confidence in the model, as a correlation between both types of data affirms the model accuracy. It is essential that a model captures and incorporates the essential details of reality to effectively calculate what is intended, particularly when modelling the open environment. Through subsequent comparisons of the model predictions with measured data, deficiencies within the model can be identified and be improved upon. For example the model may not incorporate a particular unknown vector to exposure, and measured data may aid in shedding light on this.

CASE STUDY – Monitoring data and modelled estimates of environmental 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99)

The RAIDAR evaluative model was used to predict concentrations of PBDE-99 in different environmental compartments, based on a presumed representative, per-capita emission rate. It was assumed that all emissions were a result of volatilisation to air of PBDE-99 from in-use products.

The modelled data were found to be in very good agreement with the measured data. Variances between the two sets were attributed to limitations with the assumed emission pathway, an underestimated soil degradation half-life, and the fact that nondetect monitoring data were not incorporated.

A sensitivity analysis was conducted, which revealed that the input parameters with the greatest weight on the output of the model were the biotransformation half-lives. This was to be

expected, as other chemical transformation processes are generally unlikely in the case of non-polar, neutral compounds. Uncertainty in the emission estimates of the substance also has a large impact on the predicted body burden of the model. Therefore the sensitivity analysis was crucial in determining where investigation and refinement of uncertainties would best improve the predicted body burden of the substance in humans.

This example very effectively outlines the potential for using models in exposure assessments, the mutual relationship demonstrated between modelling and monitoring data, and how both types of data can be compared to the other to refine and improve overall confidence in both.

2. Confronting uncertainty

Uncertainty and sensitivity analysis can be conducted on an exposure model to identify which processes and parameters contribute most to uncertainty. This will improve the accuracy of the exposure prediction and the risk profile. Sensitivity analysis is the process of observing the change in the outcome of a model by slightly altering one of the input parameters. This will identify the most sensitive parameters to the outcome of the calculations, hence those parameters that must be well characterised. Uncertainty lies within the range of possible outcomes of the model based upon the range of inputs that can be entered with confidence, indicating the likelihood of the predictions being accurate.

A common method is the Monte Carlo uncertainty analysis, where input parameters are assigned a distribution and randomly sampled to give a corresponding distribution of outcomes. A rank-correlation analysis of outputs against inputs of a large number of these Monte Carlo realisations can be incorporated to derive information about the sensitivity of the inputs.

3. Benchmarking of candidate POPs against existing POPs

Usually, a known PBT will have had the criteria confirmed by a number of data sources in a weight of evidence approach in order to reduce overall uncertainty. However, for candidate PBT/vPvB/POPs, where data are lacking, other methods may be employed in order to make a more confident determination of PBT properties. One such technique is the use of benchmarking, whereby key properties of known PBTs where criteria of P, B and T have been resolved more conclusively are compared with the same properties (not necessarily conclusive of P, B or T) of a candidate PBT/POP. In this way structural, physico-chemical properties, environmental fate or toxicological similarities can be established between the known PBT/POP and the candidate PBT/POP to give an indication of the likely conclusion for the desired property in question, which will have better evidence to support it than if the candidate PBT/POP was simply observed alone. The larger the benchmarking database, the greater the degree of certainty applies in this respect. A benchmarking exercise can be further reinforced by conducting statistical

uncertainty and sensitivity analyses (such as Monte Carlo analysis) which further reduce uncertainty and establish which of the inputs are the most sensitive with respect to model results (Cowan-Ellsberry *et al*, 2009).

The OECD expert group on P_{ov} and LRTP assessment (Klasmeier *et al*, 2006) recommended a benchmarking tactic when they were given the task of developing an approach for assessing whether candidate POPs fulfil the criteria of overall persistence (P_{ov}) and long-range transport potential (LRTP). They compared the P_{ov} and LRTP of the candidate POPs with that of a list of already known POPs to see if the candidate POPs were similar. A similar approach could be employed to establish whether exposure to a candidate POP would be similar to that of known POPs when emission rates are similar.

In risk assessment, the linear relationship between exposure and emission rate could be exploited to estimate the exposure of a candidate POP relative to an existing POP. This would be done by multiplying the ratio of the pseudo-intrinsic exposure metric by the likely ratio of the emissions of the candidate POP to the established POP. This is then multiplied by the ratio of the two POPs' toxicities to obtain the relative risk:

$$\text{Relative risk} = (X_{cand}/X_{known}) \times (E_{cand}/E_{known}) \times (T_{cand}/T_{known})$$

where: X is the exposure metric
E is the global emissions
T is the toxicity metric.

CASE STUDY – Benchmarking exercise

A benchmarking procedure was conducted. It compared the human body burden potential of several established POP and non-POPs to the candidate POPs for addition to the Stockholm Convention in 2006 and 2007 (Cowan-Ellsberry *et al*, 2009). Two fate and exposure scenarios were considered: One for the source region and one for a remote region 2500 km away. The information derived by these two scenarios could be compared to toxicity values to provide a screening-level risk assessment. The physico-chemical property data used in the benchmarking procedure are $\log K_{ow}$, $\log K_{aw}$, $\log K_{oa}$, and air, water, soil, sediment, fish, and avian / mammal transformation half-lives. Two models are used in the benchmarking exercise: RAIDAR and The Tool.

Tables are presented showing the predicted body burdens of each of the substances in the benchmarking exercise, both with and without biotransformation being taken into account. This is done to illustrate the effect on the results of the calculation that biotransformation has, and it is substantial.

The case study illustrates that all the investigated candidate POPs had a similar predicted body burden and long-range transport potential to the established POPs, based on the benchmarking exercise.

4. Predicting chemical exposures from emissions
If the source-receptor relationship is fully quantified, chemical fate calculations and food chain bioaccumulation can be combined to derive chemical exposure. This relies on well characterised information on emission rates, environmental characteristics, exposure pathways and receptor populations. This has been achieved for some contaminants, such as the polychlorinated biphenyls, which have been studied in detail. Many risk profiles will need to rely on several assumptions based on these variables, due to limited information.

5. Forecasting exposure in the future
Models can be used to extrapolate emissions, physico-chemical and monitoring data to forecast exposure in the future. For example, increasing measured concentrations in an environment can be used to forecast the rate of increase based on future emission scenarios, which will aid in prioritising management options.

Non steady-state modelling tools may be required in risk profiles to predict recovery times in target receptors. This is because the problems of POP exposure may take an unacceptably long time to rectify in the event that a key aspect of hazard assessment is not taken into account. A benchmarking approach, as discussed earlier, using the response time of a sentinel organism in a remote region may be employed to rectify this. This has been performed for HBCD (Arnot *et al*, 2009, see section 3.1.3.7).

6. Scenario testing of alternative risk management options
Risk management options involving different levels of future emissions or modified exposure pathways can be modelled to estimate their effectiveness. This can supply information not only on the level of changes in exposure estimated, but the rate at which they will occur.

4.2.2.2 Model predictions of levels of PBT/vPvB chemicals in organisms including humans

Czub and McLachlan (2004a,b) have developed a model (ACC-HUMAN) to describe bioaccumulation of lipophilic organic compounds from air, water and soil in humans, also linking it to a marine and agricultural food chain model to include uptake via primary dietary sources such as fish, dairy products and beef. The model is adapted to Northern European conditions, but can be modified to cover other regions as well. The model is based on the

fugacity approach and each link in the food chain is treated as one or several homogenous compartments, while each compartment comprises a mixture of several phases (e.g. water and lipid for mammals) that are assumed to be at equilibrium with each other. Each food chain compartment is interconnected with one or more abiotic compartments in the environment (air, water, soil). From this concept a number of differential equations are derived that are solved stepwise based on a set of initial conditions and boundary conditions defined by environmental parameters and chemical fugacities in the abiotic compartments. The human tissue compartment model is a two-compartment model that does not take into consideration kinetic aspects of distribution in the body. The model considers uptake via diet and inhalation and elimination via metabolism, percutaneous excretion, digestive tract excretion, exhalation, childbirth and nursing. In the model a human is born every 10 years and breastfed for six months. Child bearing intervals for women can be defined by the user. Body weight and lipid volume increase during pregnancy are accounted for and gradually reduced after birth to pre-birth values after 6.5 months. Concentrations in individuals up to 80 years old are simulated. The model was evaluated using PCBs and the environmental data for southern Sweden due to the availability of detailed information on the Baltic Sea drainage basin. The results were compared to existing biomonitoring data and the agreement was reported to be good. Input data for the chemicals are physico-chemical data, in particular the solubility in octanol or the octanol-water, octanol-air and air-water partitioning coefficients (Czub and McLachlan, 2004b) and the total quantity of the chemical in the environment of interest normalised to the surface area of that environment.

Alonso *et al* (2008) presented a model for estimating the potential biomagnification of chemicals in a generic food web based on kinetic models covering species belonging to different trophic levels. The model consists of four steps. The first step represents the accumulation in primary producers and via non-food exposure routes, mainly bioconcentration from water or exposure from sediment. The oral exposure route is considered for all consecutive trophic levels. Step 2 links individual models for the food chain with one species in each level. Step 3 segregates each level into several species and can cover different exposure patterns. The fourth step establishes complex relationships among species and defines the position of each species by the Trophic Index that is based on the feeding preferences of predators. The model was mathematically implemented through system dynamic models and Monte Carlo based probabilistic calculations using crystal ball. The model was validated for PCB 153 against a well-documented food chain of the Barent Sea as described by Hop *et al* (2002) and a good correlation was reported. The model can be run at different levels of complexity at different tiers of the risk assessment and could constitute a valuable tool for a refined assessment of biomagnification and exposure for different organisms.

4.2.3 Monitoring Data

Exposure assessment is an intrinsic part of risk assessment, and measuring concentrations of substances in different environmental media is the most reliable way of affirming and quantifying suspected exposure. ECETOC (2005a) provides an overview of the use of monitoring within a high tier risk assessment. This includes a number of information sources that can be referred to for detailed advice on the design and execution of environmental monitoring programmes. As mentioned previously with regard to the use of models to estimate environmental exposure, the use of models in tandem with monitoring programmes can be powerful in both targeting monitoring resources, as well as validating and strengthening models used to predict environmental exposure.

4.2.3.1 Abiotic monitoring

Exposure assessment programmes

There are three types of exposure assessment programmes:

- Monitoring programmes – large scale, long-term programmes which are generally carried out by government institutions.
- Surveys – sampling programmes of finite duration. They can be used in conjunction with monitoring data to support extrapolation to other substances / species not currently covered by the monitoring programme. These are likely the most feasible programmes that will be utilised when conducting a targeted exposure assessment to fill a data gap for risk assessment.
- Surveillance – repeated surveys designed to detect a temporal change in observations.

Guidelines for using new measurements to demonstrate exposure of candidate chemicals of concern

In designing a new measurement programme to provide evidence which demonstrates exposure to new and candidate chemicals of concern (e.g. for a risk assessment), considerations must be taken to ensure that the data are obtained most efficiently and is most useful to the purpose. This is because a detailed and labour intensive monitoring program, like those funded by government institutions, will usually not be feasible. A database (MonitoringBase) of ongoing and planned monitoring programmes was prepared under CEFIC LRi funding (MonitoringBase, freely available on CD from CEFIC, Brussels). This should be a useful and cost-effective way of identifying appropriate monitoring programmes that can be used when specific chemical measurement exercises are needed to support exposure predictions or risk assessments. UNEP has published thorough guidance for sampling, quality assurance and analytical methods for the

legacy POPs (UNEP, 2004). These can also be applied in designing measurement collections for candidate POPs or PBT/vPvB substances.

Case Study – Cyclic Siloxanes in the Environment

The cyclic siloxanes D4 (octamethylcyclotetrasiloxane), D5 (decamethylcyclopentasiloxane) and D6 (dodecamethylcyclohexasiloxane), known as volatile methyl siloxanes (VMSs), were identified as potential vPvBs. Due to these concerns a robust monitoring programme has taken place to measure VMS concentrations in a range of environmental compartments. This programme, coordinated by the Silicone industry (Centre Européen des Silicones, or CES), was used to assess the validity of the output from environmental distribution modelling which indicated that VMSs would mainly partition to air. This monitoring programme is detailed within the UK Environmental Agency reports on D4, D5 and D6 (e.g. Brooke *et al*, 2009). Further industry activities to monitor environmental concentrations of VMS in air, sewage effluent, river water, sediment, and biota are underway. This includes investigation of the persistence and bioaccumulation of VMSs in the field taking into account time trends and spatial distributions in a number of environmental compartments and remote locations (e.g. Brooke *et al*, 2009):

- Historical deposition patterns in lakes to evaluate degradation in the environment.
- Continuation of a mussel-screening program.
- Study the distribution and persistence downstream from a known point source (monitoring and river die-away study) within example EU river.
- Site-specific monitoring to refine the PECs used in the risk assessment.

These activities are supported by ongoing analytical methodology development for the different environmental media.

Case Study – Measuring and monitoring POPs in the context of risk assessment

Wu *et al* (2008) discussed the factors involved in taking environmental measurements of POPs in order for them to be meaningfully used in risk assessment. 661 environmental POP measurement reports, published since 1990, were reviewed. Of those reviewed 304 reported concentrations only at a single spatial and temporal point, or samples that were pooled for analysis. Of the remaining 346, 290 reported spatial distributions, 29 reported temporal changes and 27 reported both temporal and spatial changes. Few of them made references to the environmental consequences of their measurements. This meant that there would be difficulty in establishing reliable conclusions in the temporal and spatial variability of POP concentrations.

Another concern was the number of replicates that were taken in most of these studies. Sufficient replicates must be taken in order to give statistically valid and reliable estimates of the field concentrations at a particular spatial or temporal point, and to discriminate the differences between sampling sites and times, to prevent erroneous conclusions. There was a great variability in measurements taken between the replicates of nine marine POPS monitoring studies, and the previously reviewed reports, 299 did not take any replicates. Using the variance between the 9 reported monitoring programs, power analysis to determine the probability of detecting a 20% difference between site and/or time using 2, 5 and 10 replicates was performed. For 2 replicates, the probability ranged from 3 to 33%. For 5 replicates, the probability ranged from 4 to 40% in 8 out of the 9 cases, and was over 50% in one. For 10 replicates, the probability ranged from 5 to 28% in 5 cases, and was above 50% in the remaining 4 (highest was 96%). This highlights the imperative nature of sampling a sufficient number of replicates to obtain statistically reliable results.

4.2.3.2 Biotic monitoring

The exposure process of a chemical begins with a source of emissions, followed by the chemical moving through the environment through transport and fate processes. The species of concern then comes into contact with the environmental media containing the chemical, resulting in exposure. Following this initial exposure are the pharmacokinetic processes by which the substance is absorbed, distributed, metabolised and eliminated in the organism. The amount of substance which reaches the absorption step, the first pharmacokinetic process, is known as the 'absorbed dose'. The substance will travel through the organism and migrate to its receptor, binding to it and becoming biologically active. The amount of the chemical which arrives at this final point is known as the 'biologically effective dose'. The further along an exposure route a measurement is taken, the higher the degree of certainty in establishing the biologically effective dose in the receptor (Swackhamer *et al*, 2009).

It is normally impossible to assess the biologically effective dose of the substance of concern, so the next most reliable stage to measure the exposure is the dose reaching the general circulation (internal dose). This is most frequently done in human biomonitoring studies using low invasive techniques such as via determination of the concentration in blood (blood plasma or serum) or breast milk. With an adjustment of the lipid content in each medium, the concentrations are frequently similar. The use of breast milk concentrations in an assessment can provide an indication of the oral exposure to an infant via breast feeding. [Average daily breast milk intake as a function of age has been determined by Arcus-Arth *et al* (2005) for example]. Milk concentrations can also give an indication of historical maternal exposure, because lipophilic chemicals are present in higher concentrations in lipid than in blood, and milk can be obtained by

non-invasive methods. However, the high lipid content and complex composition can also complicate the analysis in mothers' milk.

Biomonitoring data may provide a more relevant estimate of exposure as they integrate all routes and sources of exposure and also the toxicokinetic behaviour. Biomonitoring data may also be used to verify mathematical models that are used to predict human exposure. External sources of exposure can be related to the biomonitoring data and may also be helpful to determine the contribution of concentrations in different environmental compartments (e.g. air, water, food chain) and emission sources to the body burden. Biomonitoring may also provide information on the body burden of different sub-populations and thus enable a refined risk assessment for possibly susceptible sub-populations (ECETOC, 2005b). If biomonitoring of the same populations is continued over several years it may contribute to assess if there is an increasing trend with time and if this can be related to the properties of the substance (e.g. as an indication of the biomagnification potential) or the increase of external exposure. Time trends can also be useful to assess the response time to exposure controls and the reversibility of internal exposures. The usefulness and interpretation of biomonitoring data for risk assessment is dependent on the quality of the sampling and analytical methods, the characterisation of the external exposure situation, the existence of a suitable biological matrix and suitable parameters that are able to reflect internal exposure, the characterisation of the sampled population and reliable reference and limit values from the hazard assessment to enable a comparison of the internal exposure with respective hazard data. A discussion of these factors is provided in IPCS (2001); ECETOC (2005b); Albertini *et al (*2006); and Angerer *et al* (2007).

In a recent publication, Den Hond *et al* (2009) studied methodological implications for human biomonitoring using data of the Flemish Environment and Health study that was conducted between 2002 and 2006 and determined marker polychlorinated biphenyls (PCB 138, 153 and 180), hexachlorobenzene (HCB) and p,p'-diphenyldichloroethylene (p,p'-DDE) in serum samples of 1196 newborns and their mothers, 1679 adolescents (14 to 15 years old) and 1583 adults (50-65 years). The authors identified the factors that contributed most to the variability of the data as blood fat, age, sex, body mass index, change in body weight, area of residence and local meat consumption.

This study is a good example of the possibilities and limitations of the uses of biomonitoring data for risk assessment purposes. The levels observed can be related to adverse effect levels, but other factors apart from external exposure concentrations are influencing the measured concentrations and clear quantitative correlations between internal and external exposures are almost impossible to obtain for the general population even with a well-designed approach to capture possible covariates. To prioritise those influences and assess the risk for different subpopulations the multi-variant analysis as performed by the authors may be useful. Repeated

biomonitoring studies to establish time trends need to be carefully designed in order to get results that can reasonably be compared taking into account the most important determining factors.

When assessing exposure to higher trophic levels in wildlife it is important to have a reliable assessment of the biomagnification in the food web in which the species belongs, as this will aid in establishing the fine details of the route of exposure. Well studied food webs include the Great Lakes pelagic food web, the Baltic Sea marine food web and the lichen-caribou-wolf food web. They also allow trophic magnification factors (TMFs) to be established and assessed.

A single, surrogate, biomonitor species is generally chosen, which will be representative of a wide range of top predators, allowing the exposure to be more broadly established. The biomonitor species is required to have:

- Demonstrated accumulation.
- Ease of collection.
- Availability of samples.
- A long lifespan, with samples being taken from the optimum of this lifespan.
- Well-documented feeding habits.
- Limited migration.
- A tolerance to a range of environmental and climatic conditions.
- A well-known life history.
- Commercial and economic importance in the area.
- Ability to be transported internationally without legal implications.

It is argued that, in order for the results of monitoring studies to be interpretable and meaningful, they must be obtained based on the context of risk assessment. A risk assessment-based example of a monitoring program was presented with the case of polychlorinated biphenyls in the green lipped mussel (*Perna virvidis*). Lysosomal integrity was found to have an impact on a number of different processes and qualities of a eukaryotic cell, and is known to correlate with cellular wellbeing. *Perna virvidis* were sampled from various locations in Hong-Kong and both lysosomal integrity and internal PCB concentration were determined revealing a significant correlation. A NOAEL (< 200 µg/kg lipid) and LOAEL (200 µg/kg lipid) were determined, and it was assumed that these would be similar for similar species in different locations. Taking these effect concentrations as threshold of biological concern, they were compared to PCB levels measured in other studies conducted in other areas and determined that mussels in Japan, the Baltic Sea and Italy should be considered ‘at risk’. This demonstrates how measurements can be performed and interpreted in a much more meaningful way (Wu *et al*, 2008).

4.2.4 Food chain / Bioaccumulation

4.2.4.1 Biomagnification Factor (BMF)

Currently, there is no scientific definition for bioaccumulation potential in the different regulations that deal with PBT chemicals, adding considerable uncertainty to the determination of the 'B' property. Two different processes are recognised to contribute to chemical bioaccumulation. They are bioconcentration (i.e. chemical exchange between the respiratory medium and the organism) and biomagnification (i.e. chemical magnification from dietary ingestion). Chemicals with the greatest capacity to bioaccumulate are those subject to both bioconcentration and biomagnification. These two parameters can be determined using field data or standardised test methodologies. The most well-known test is the OECD 305 fish bioconcentration test, which will produce a Bioconcentration Factor (BCF). Unfortunately, this method is of limited use for poorly water soluble chemicals. As stated in the ECETOC Technical Report No 98 (ECETOC, 2005a), determining biomagnification using dietary exposure experiments is a valuable methodology for poorly water soluble substances for which an OECD 305 test is not feasible. The gastrointestinal magnification process that causes chemical biomagnification in food webs will be shown in dietary experiments if the rates of chemical elimination and metabolic transformation are low (Gobas *et al*, 2009). To obtain reliable results in dietary biomagnification tests, chemical concentrations in the predator (test organism) and prey (feed) have to be expressed on a common basis. The most common normalisation is performed using lipid concentrations, but other methods are available, such as the use of chemicals fugacities or normalising chemical concentrations using the lipid, protein, carbohydrate and water fractions in the organism, which is more relevant than using lipids alone (Gobas *et al*, 2009).

A chemical is considered to biomagnify in the food chain if its Biomagnification Factor (BMF) is significantly greater than 1. Attempts have been made to calculate the BCF from experimentally determined BMFs, because BCF is a parameter that is often used in regulatory schemes dealing with PBT evaluation. However, recalculation should be avoided because the results are highly uncertain. When available, the BMF can be used on its own as a prioritisation tool to determine if a chemical is likely to bioaccumulate. Currently, a ring test is being performed to determine whether the dietary bioaccumulation protocol should be included in the next revision of the OECD 305 bioaccumulation guideline.

4.2.4.2 Trophic Magnification Factor

BCF or K_{ow} do not take into account the magnification of contaminant levels up trophic levels in a food web (Gobas *et al*, 2009), which shows the true bioaccumulation of a substance. The trophic magnification factor (TMF), or food web magnification factor (FWMF), is proposed as

the recommended method of quantification of a substance's ability to biomagnify through the ascending trophic levels of a food web. It is defined as the average factor by which the normalised chemical concentration in biota of a food web increases per trophic level (Gobas *et al*, 2009). It is determined from the slope (*m*) derived by linear regression of logarithmically transformed normalised chemical concentration in biota and trophic position of the sampled biota. The TMF for a chemical incorporates bioconcentration (i.e. exchange between respiratory medium and organism), biomagnification (i.e. magnification by dietary ingestion) and biotransformation (i.e. transformation of the chemical through metabolic processes).

$TMF = 10^m$ (A TMF > 1 signifies a substance that is biomagnifying as it ascends a food chain.)

In order to determine a TMF, normalised concentrations of a substance in the biota from more than one trophic level must be measured. The normalisation process will account for the differences in organism body composition (such as lipid content) of the different trophic levels. This factor is one of the major uncertainties in TMF determination, because seasonal and species specific lipid content dynamics can greatly influence the calculation (De Laender *et al*, 2010; Kelly *et al*, 2009). Trophic positions can be assigned by performing analyses of the intestinal contents of organisms, followed by the assignment of a numerical value by a trophic positioning model. An alternative approach is to detect the ratio of N^{15}/N^{14}, as this is known to increase with ascending trophic position.

TMFs are likely to differ between aquatic and terrestrial food webs as the mechanisms of bioaccumulation are likely to differ between water and air breathing organisms. An example of this is a study by Kelly *et al* (2007), where biomagnification (TMF > 1) of organic contaminants with moderate octanol–water partition coefficients (K_{ow}) but high octanol–air partition coefficients (K_{oa}) was observed in terrestrial food webs due to airborne exposure, while in aquatic food webs no biomagnification of these substances was observed due to rapid elimination.

The TMF can be invaluable in exposure assessments of PBTs and POPs. When measured data are available for the concentrations of a contaminant in an organism of a lower trophic level in a known food chain, the likely tissue concentrations of organisms of higher trophic levels in that food chain can be determined by using the TMF. This would require information on the trophic levels of the species and extensive knowledge of the food web framework, such as the dietary proportions in which the lower species is ingested by the higher one.

In the identification of new and candidate POPs, a benchmarking model may be developed (in the manner discussed in Cowan-Ellsberry *et al*, 2009) to assess the likely TMF of these substances, based on known TMFs of other POPs and their chemical properties (see 'modelling exposure.doc'). At present, bioaccumulation models have the capacity to calculate TMFs based on the chemical properties of the substance and include algorithms which calculate biological and

environmental effects on the degree of bioaccumulation. These factors include: Membrane permeation, bioavailability, faecal excretion, temperature, organic carbon content of bottom and suspended sediments, among others. They also include several classes of organisms. As a screening exercise, using trophic magnification factors would be invaluable in cutting down a list of potential chemicals for assessment (for example in designing a tiered risk assessment), as TMF is a conclusive determinant of bioaccumulation potential and those substances with TMF <1 would not be of concern in this regard.

Case studies

The trophic magnification factor of triphenyltin (TPT) in a marine food web was measured and compared with that of tributyltin (Hu *et al*, 2006). Samples of primary producers, five invertebrates and six fish, as well as surface water and sediment, were collected. Lipid content was determined gravimetrically. Trophic levels (TL) were calculated based on stable nitrogen isotope ratios using the following equation:

$$TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton})/3.8$$

The TMF was calculated based on the relationship between TL and the organotin concentration using linear regression:

$$\text{Log (organotin concentration)} = a + b\text{TL}$$
$$\text{TMF} = 10b$$

It was found that the internal concentrations of TBTs decreased with increasing trophic level, and the TMF was estimated to be 0.59. Significant trophic magnification was indicated for TPTs with a TMF of 3.70. Least squares regression was performed ($p < 0.001$) and indicated high statistical significance. This TMF was similar to that calculated for DDE and HCB in the same food web, which suggested that TPT would biomagnify to a similar extent to organochlorines. This high level of biomagnification was thought to be due to the low rate of metabolic transformation of TPT. TBT has a higher log K_{ow} than TPT, hence would be predicted to be more bioaccumulative than TPT using conventional K_{ow} considerations. The log K_{ow} of TPT is 3.5, which is lower than the limit value of 4.5 for screening substances for their bioaccumulation potential.

Several examples of TMF values are available for PAHs. Field data provide evidence that PAHs are not bioaccumulative with increasing trophic level in three food web biomagnification studies (Wan *et al*, 2007; Nfon *et al*, 2008; Takeuchi *et al*, 2009). Slopes of log lipid-normalised PAH concentration versus trophic level for nineteen different PAHs are available. Statistically significant positive slopes demonstrate evidence for biomagnification while zero or negative slopes provide evidence of no biomagnification or trophic dilution. For all PAH investigated in

these three studies, no significant positive slopes were observed. TMFs were calculated as the anti-logarithm of these slopes and are reported in Table 7. All 38 TMFs are below one except two values that are not statistically different from one. Thus, field studies collectively support that PAHs do not biomagnify but instead may undergo trophic dilution.

Table 7: Trophic magnification factors for the marine food webs in Bohai Bay, Baltic Sea and Tokyo Bay. Antilogs of the slopes of the regression equations for the lipid-based PAH concentrations versus $\delta^{15}N$ were used to calculate TMFs

Compound	TMF (Wan *et al*, 2007)	TMF (Nfon *et al*, 2008)	TMF (Takeuchi *et al*, 2009)
Acenaphthylene	0.45		
Acenaphthene	(1.02)		
Benz[a]anthracene	0.2	0.75	(0.83)
Benzo[a]pyrene	0.24	(0.75)	(0.80)
Benzo[e]pyrene	0.25	(0.86)	(0.57)
Benzo[b]fluoranthene			0.60
Benzo[b+k]fluoranthene	0.27		
Benzo[j+k]fluoranthene			0.69
Benzo[k]fluoranthene		(0.84)	
Benzo[ghi]perylene	(0.66)	(0.75)	(0.72)
Chrysene	0.26	0.66	0.65
Fluoranthene	0.11	0.72	0.60
Fluorene	(1.15)		
Indeno[123-cd]pyrene	(0.81)	(0.75)	(0.80)
Dibenz[ah]anthracene	(0.85)		
Perylene	0.24	(0.67)	(0.77)
Phenanthrene	(0.43)	0.82	0.75
Pyrene	0.17	0.74	0.62

Values in parenthesis denote that slopes of lipid normalised concentrations versus trophic level using in calculating TMFs were not significantly different from zero.

4.2.4.3 Probabilistic exposure (risk) assessment

The results of these studies outline the value of trophic magnification factors in predicting bioaccumulation potential of substances. Conventional means of comparing the octanol-water partition coefficient to a threshold value do not take into account factors such as biotransformation. In this case, the TPT substance would not be predicted to be bioaccumulative through these conventional means, while the TMF value that was measured gives strong evidence not only that it is bioaccumulative, but also that it is to a certain extent similar to persistent organochlorines. On the other hand, TMF studies show the lack of biomagnification for substances that are actively biotransformed, like PAHs. TMFs allow a better understanding of the impact of bioaccumulative substances in the food web; however, more ecological research is

needed to elucidate the variation in TMFs in different environments (e.g. lentic versus lotic, Walters *et al*, 2008), mainly caused by the uncertainties in food web composition and the uncertainties around contaminant uptake, transformation and elimination.

Risk assessment methodologies that are deterministic in nature (e.g. EU TGD [EC, 2003]) include many sources of uncertainty which can be based on Forbes and Calow (2002):

- A lack of understanding or environmental realism which, in principle, can be reduced through the generation of further, relevant, information.
- True variability which is an inherent property of the environment which cannot be reduced but can be accounted for within probabilistic risk assessment methods.

For substances that are categorised as PBT a high tier risk assessment strategy can be designed in order to address a lack of understanding or environmental realism. There is also the opportunity to adopt probabilistic risk assessment methodologies which can better account for the uncertainties inherent within an exposure, food chain or effects assessment of a chemical. Such probabilistic approaches are becoming more common place within chemical risk assessment (e.g. Species Sensitivity Distributions) and an example of how they have been applied to a case study substance from this report is provided below.

An ecological risk assessment of HCB to mink was conducted in Canada (Moore *et al*, 1997). This study employed both a highly conservative quotient method and a Monte Carlo simulation to probabilistically assess the risk of adverse effects. The process began with problem formulation, where a scoping exercise was conducted to identify the receptor of concern (i.e. the mink), which needed to be a highly exposed and sensitive species. A conceptual model of the exposure route was also assembled. The mink was chosen because, as an organism, it possesses many of the characteristics that make it a suitable surrogate species (see section 3.1.3.8).

Exposure was characterised by collaborating measurement data of HCB in air, water and fish as the key component of the mink's diet. Effect characterisation was based on studies conducted for the reproductive effects of HCB on mink.

The quotient method of risk characterisation involved the development of hyper-conservative point estimates of daily intake of HCB through air, water and fish, based on the measured concentrations. These concentrations were based on the maximum reported concentrations and the mink's ingestion rate was in the 95% upper confidence limit of normal mink. The total daily intake (TDI) was defined as the sum of the three intake concentrations (air, water, fish):

$$TDI = C_{air}NIR_{air} + C_{water}NIR_{water} + C_{fish}NIR_{fish}$$

where C is the concentration of HCB and NIR the intake rate normalised to 1 kg wild female mink.

A safety factor of 0.1 was applied to the lowest effects dose of 0.16 mg/kg body weight to derive the estimated no-effects dose and account for differences between laboratory and field studies. These point estimates and safety factor values were designed so that, even though there would be a high degree of uncertainty in the assessment, a conservative threshold would be produced, below which there was a very low probability of adverse effects.

Through this risk assessment, risk quotients exceeded 1 for the St. Clair River and Lake Ontario areas. All other assessed areas produced risk quotients below 1. From these results it can be interpreted that the risks of adverse effects cannot be ruled out for those areas with RQs > 1. But as this assessment used conservative exposure and effect parameters, it was unlikely that this was a real estimate of the risk. As a screening approach, the assessment could be used to exclude the other areas from further risk considerations as there was no indication of significant risk, using the very conservative conditions.

The Monte Carlo simulation of risk involved calculating a probability distribution of exposures and plotting a cumulative dose response relationship (with 95% confidence intervals), based on the effects data. When these two distributions were combined they gave a probabilistic risk function for the effect of HCB on reproductive fecundity.

The equation used to define TDI in the quotient method assessment was expanded to better assess the contribution of the mink's diet to exposure.

$$TDI = C_{air}NIR_{air} + C_{water}NIR_{water} + \frac{C_{fish}P_{fish}MR_{fw}}{AE_{fish}GE_{fish}} + \frac{C_{crus}(1-P_{fish})MR_{fw}}{AE_{crus}GE_{crus}}$$

where:

C is the concentration
NIR is the intake rate normalised to 1 kg wild female mink
P_{fish} is the proportion of fish in the diet of wild female mink
AE (unitless) is the assimilation efficiency for mammals eating fish (fish) or crustaceans (crus)
GE (kcal/g) is the gross energy of fish or crustaceans
MR_{fw} (kcal/d) is the metabolic rate of wild female mink.

Probability density functions (PDFs) were assigned to each of these variables (apart from those that were not supported by sufficient data and air and water NIRs, as they were expected to have minimal impact on the overall risk). 10,000 Monte Carlo simulations were run and Latin Hypercube Sampling was used to ensure adequate sampling from all portions of the input PDFs.

The results of the risk assessment were plotted as reverse cumulative probability (%) over decline in reproductive fecundity (%). An example of the result is the effect dose for a 20% decline in reproductive fecundity was 59,999 ng/kg body weight/d. A sensitivity analysis of the assessment revealed that key input PDFs were metabolic rate, gross energy of fish and concentration of HCB in fish. The conclusions of the two risk assessments were that the only location of concern for mink in the Great Lakes region was a short stretch of the St. Clair River shoreline.

4.3 Effects

As described earlier, effects considerations are equally important to exposure estimations in risk assessment. Often in the case of PBT/vPvB/ POPs, the substance will act by a non-specific mode of action in order to cause an adverse effect. Also, due to the bioaccumulative nature of these substances, receptors found to be the most sensitive are often top predators, where biomagnification has caused what initially did not appear to be dangerous concentrations of the substances in biota to increase up the food chain to dangerous levels. Toxicity data for substances in species at these upper trophic levels are far more difficult to come by and further testing carries with it heavy ethical and legislative restrictions. Hence, more specific techniques are necessary in order to confidently establish adverse effect levels for the purposes of risk assessment. These include tissue residue and total daily intake (TDI) approaches. The tissue residue approach establishes a residue-based PNEC using known effect levels from previous testing of the substance. This may then be directly compared to real tissue monitoring data to obtain a direct indication of the possibility of adverse effects. The TDI approach compares dose NOAELs from observed experimental data to the estimated intake rates of target species in the environment.

ECETOC (2005a) included a number of factors considered most relevant in affecting the uncertainties associated within the effects assessment of chemicals categorised as PBT as well as a number of refinement options that could be progressed to reduce these uncertainties. The section below builds on ECETOC (2005a) by elaborating further on refinement options mentioned in that report as well as identifying additional refinement options that could be considered within a high tier risk assessment strategy for a substance categorised as PBT. These refinement options have been selected based on the opinions of Task Force members of their relevance within a risk assessment strategy for substances as well as their application within case studies included within Chapter 3.

By identifying these refinement options as potentially relevant within a risk assessment strategy for PBT substances it is assumed that information relevant for lower tier effects assessment are already available and have been generated to a high quality (e.g. correctly accounting for poor water solubility that can be expected for chemicals categorised as PBT, for test duration to ensure

steady state is reached within the organism and more appropriate routes of exposure such as dietary versus exposure via water column [ECETOC, 2005a]).

4.3.1 Case studies

These approaches were used in the assessment of HBCD, as standard toxicity tests had proven inconclusive due to the substance's very low water solubility. One toxicity test had observed a chronic NOEC < 10 µg/L; however there was concern that the use of a solvent carrier had artificially increased the solubility of HBCD and that, under environmental conditions, this concentration would not be achievable. The tissue residue approach adopted two PNECs: A baseline narcosis and a specific mode of action (worst case) PNEC. From this assessment it was deemed that some tissue concentrations in biota approached the 'worst-case' PNEC level, representing a possible concern. The TDI approach however did not indicate any risk for piscivorous marine mammals in all geographic locations, as TDIs were estimated to be well below measured NOAELs.

TBTO is another example where refined effects assessments were used. For the environment a specific mode of action and a sensitive receptor were identified and a PNEC derived from this assessment that considerably reduces the uncertainty of the environmental risk assessment. Also a specific toxicological endpoint and a mode of action were identified in the human health risk assessment for tributyltin compounds. The dose response was well characterised and provided a reduced level of uncertainty for the risk assessment when the TDI levels were compared with high quality monitoring and modelling exposure data.

4.3.2 Use of mode of action to inform testing strategy

Environmental Risk Assessment

In the process of developing standardised protocols, the environmental toxicology community have settled on sentinel organisms (e.g. algae, crustaceans and fish for 'base set' assessments) as surrogates for the wide range of taxa found in aquatic ecosystems. It is often based on these data that the 'T' designation is conferred. Given the subtlety of potential effects and the diversity of species possibly affected, ECETOC (2007b) proposed the application of Intelligent Testing Strategies (ITS) to target those most likely to be impacted thereby improving the use of resources and reducing uncertainty introduced through extrapolation.

The strategy proposes that a protein target mode of action (MOA) approach has significant potential to inform read-across approaches for chemical classes, support the further use of *in vitro* hazard assessment tools and can usefully guide the selection and design of *in vivo* aquatic toxicity

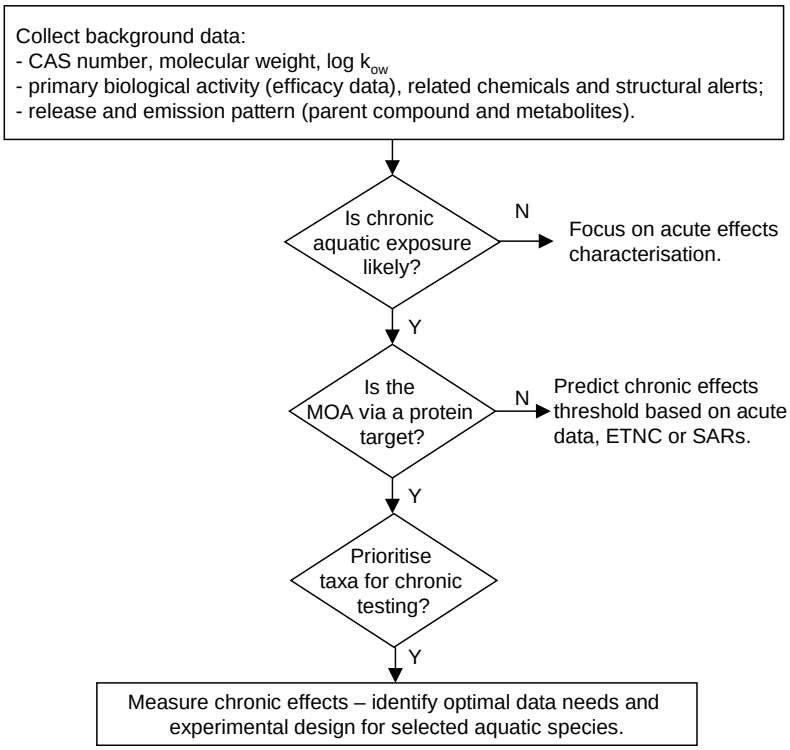
tests. This approach builds on the Verhaar *et al* (1992) categorisation system which separates chemicals into classes that can be assigned a toxic MOA as follows:

- MOA1 - inert chemicals (baseline toxicity);
- MOA2 - less inert chemicals;
- MOA3 - reactive chemicals;
- MOA4 - specifically acting chemicals.

Using case studies, it was proposed that MOA4 chemicals may have toxicological effects due to (specific) interactions with certain receptors and, expanding upon published ITS schemes, developed a simple five step flow-chart (Figure 3). The recommended process includes:

1. Collection of MOA information on the primary pharmacological / toxicological activity (and any additional toxicity MOAs) for the chemical of interest for the target species as well as mammalian data for a weight-of evidence type of approach;
2. use of non-traditional sources of biological information, especially the growing biomedical and 'omics electronic databases on zebrafish, marine invertebrates and other non-mammalian species;
3. if there is evidence for the main MOA being via a protein target (e.g. enzyme or hormone receptor), this insight can be used to guide the efficient selection of regulatory test methods;
4. measurement of biomarker responses (e.g. vitellogenin) if desired for read-across purposes or setting test concentrations, however, focus should be on population relevant endpoints (survival, development growth and reproduction) for generating NOEC values or calculation of PNEC values for application in environmental risk assessment;
5. caution is indicated for the use of acute interspecies sensitivity ratios (ISRs) for algae, crustaceans or fish, since the available data suggest they are of limited value for ITS application, presumably since acute high levels exposure induces different MOAs compared to chronic low level exposures (Note: Available data suggest that MOA4 chemicals can have ISRs from < 10 to > 500, compared with ISRs for MOA1 (< 10), MOA2 (8.6 to 343) and MOA3 (< 5 to > 25).

Figure 3: Five-step prioritisation of prediction or measurement of chronic effects in aquatic species (ETNC = Environmental Threshold of No Concern; SARs = Structure Activity Relationships) (ECETOC, 2007b)



Evaluation of these data provides information on exposure to, and possible MOA of, the substance. Such insight may come from efficacy or therapeutic data in the case of pharmaceuticals, or from 'read across' within chemical classes. Concerning effects, valuable guidance may be obtained from molecular, biochemical or cellular toxic responses measured in both *in vivo* and *in vitro* studies. For example, fadrozole is an anti-cancer drug which is designed to inhibit aromatase activity in breast tissue (*in vivo* and *in vitro*) and which also decreases vitellogenin levels in female fish (and in fish hepatocytes *in vitro*).

With this approach it may be possible to confirm whether there may be merit in conducting studies to elucidate the MOA of the chemical in question to better inform an environmental risk assessment.

An environmental risk assessment conducted as part of the new drug marketing authorisation process typically includes studies extending to fish early life stage. In some cases it may be argued that, although informative, the early life stage may not be the best indicator of potential long-term effect. Chemicals with specific mechanisms of action such as pharmaceuticals could

trigger physiological changes in systems other than reproductive / developmental which would be missed.

To make best use of the extensive mammalian dataset accumulated during drug development when assessing environmental risk, there have been numerous 'read-across' theories postulated, such as the comparative plasma concentration model of Huggett *et al* (2004). The model seeks to exploit conservation of receptors across species, i.e. vertebrates, to predict potential for activity in non-target species.

In the model, an effect ratio (ER) is calculated comparing measured human therapeutic plasma concentration (HTPC) to predicted steady state plasma concentration (FSSPC) in fish, (ER = HTPC / FSSPC). The HTPC is commonly obtained early in drug development and the FSSPC can be calculated from environmental concentration and expected absorption and bioconcentration estimated from hydrophobicity (i.e. octanol: water partition). Since pharmaceuticals typically function via specific receptor binding, with enzymes and receptors similar to those in mammals conserved in fish, responses can be anticipated when the plasma concentration in fish approaches the plasma concentration in humans at which therapeutic effects are observed. The lower the ER, the greater the potential that a receptor-mediated response can be expected in fish.

Common receptors have been documented for several aquatic vertebrates (Huggett *et al*, 2003; Gunnarsson *et al*, 2008). Coupled with knowledge gained from mammalian studies investigating fertility, reproduction, mutagenicity, genotoxicity, and to lesser extent carcinogenicity, receptor-binding affinity can be used to inform targeted testing schemes in pertinent species.

Although not yet used extensively to date, the model has been corroborated in the literature (Giltrow *et al*, 2009; Huggett *et al*, 2004; Owen *et al*, 2007; 2009) and is gaining interest. Propranolol, a non-selective beta-adrenergic receptor antagonist, makes an interesting case study. Propranolol is an antihypertensive, antiarhythmic medication with a long history of therapeutic use and has been studied in chronic fish toxicity studies.

Giltrow *et al* (2009) conducted 21-day reproduction studies of propranolol in fathead minnows at nominal concentrations from 0.001 to 10 mg/L (measured concentrations typically from 78 to 130%). Reproductive endpoints were largely negative; however, a side experiment to assess predictability of the fish plasma model for propranolol proved most interesting.

"By using the 'fish plasma model', the predicted FSSPC are within 1 order of magnitude of the measured FSSPC, and hence the model does quite well in predicting the concentrations of propranolol in the plasma. These data support the fish plasma model and show that the read-across of information from humans to fish is plausible in that existing human pharmaco- and

toxico-dynamic data could be used as a starting point to predict effects on organisms in the environment (at least those sharing targets with humans).”

From a regulatory perspective, an international workshop was held on August 25, 2010, presenting and discussing results of an UBA-financed project on modelling approaches for hazard prediction of pharmaceuticals to aquatic organisms. Extrapolation approaches, such as the fish plasma model (Huggett *et al*, 2003) to predict specific effects to occur in non-target organisms, have been proposed to be used as a tool for tailored environmental risk assessment of pharmaceuticals. The workshop aimed to inform partners from research institutes, regulatory authorities and the pharmaceutical industry about project findings, to discuss the suitability of models for pharmaceutical risk assessment and to identify next steps to improve model utility. The potential for regulatory acceptance of a model to inform risk assessment submission requirements marks a significant milestone in the advancement of Intelligent Testing Strategies and recognition of the importance of mechanism of action in risk assessment.

As a better understanding of receptor binding and mechanism of action for industrial chemicals through programs like TOXCAST is developed, it may become possible to apply similar models to elucidate toxicity mechanisms to refine risk assessment.

Human health

Standard toxicity studies in many cases only give indications of the target organs and tissues of toxicity, but generally lack information on the underlying mode of action. Based on the results of standard studies it may be possible to perform targeted investigations to elucidate the mode of action and possibly come to refined hazard assessments and identify early markers of substance-related changes that can be used in a refined risk assessment approach. However, care must be taken that a clear distinction can be made between substance-related adverse effects and variations in physiological parameters within the normal range that are not considered as adverse (ECETOC, 2002; Lewis *et al*, 2002). Several new methodologies may in the future be applied to identify modes and mechanisms of action and refine hazard assessments in this way. Some of these approaches are summarised below.

ToxCast - Application of pharmaceutical high throughput screening technologies to PBT

The assertion that chemicals meeting PBT or vPvB criteria should be assumed to be harmful is primarily the result of a lack of mechanistic data. With an understanding of the interaction of chemicals with biological receptors a prediction of the potential impact of long-term (i.e. persistent) or prolonged (i.e. bioaccumulative) exposure can be better predicted.

Pharmaceuticals are highly studied compounds in commerce so a review of the applicability of the drug development process is of interest in this regard. Elemental to the discovery of a pharmaceutical is the identification of target receptors involved in the disease of interest and the use of high throughput screening (HTS) bioassays identify promising candidates by comparing binding preference (affinity and selectivity). Successful candidates are progressed through *in vitro* and *in vivo* pre-clinical and clinical efficacy and safety studies.

While data on the effects of industrial chemicals in humans are rare and it is impractical to test chemicals in animals to the extent done for pharmaceuticals, there are some developments of methods that may in the future be used in certain cases to translate *in vitro* technologies developed in drug discovery to chemical risk assessment.

In 2007, the US EPA initiated the ToxCast™ program[8] with the aim to employ more than 400 drug-development HTS bioassays to prioritise toxicity testing of environmental chemicals. HTS endpoints include protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos. The program will first profile a collection of pesticides with datasets including developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays to establish relationships between the assays results and findings from the traditional toxicity studies and identify toxicity pathways that are relevant to human health effects. The second phase will look at broader classes of chemicals to corroborate the predictions after which the program will be used within the Agency to prioritise chemicals for testing and target the pertinent endpoints to be assessed.

In Europe, ECETOC has developed a research proposal on "High information content technologies in support of read-across in chemical risk assessment" for the CEFIC Long-range Research Initiative (LRi www.cefic-lri.org) (ECETOC, 2010b). In particular, the aim is at a mechanistic concept using 'omics tools and *in silico* methods, unlike the empirical ToxCast™ approach.

The research proposal includes the systematic search for chemical analogues with available tools for building an initial hypothesis on read-across to similar substances or metabolism to substances of known toxicity. This hypothesis should then be tested by the application of further specific *in silico* models or use of increasingly available data and methods in the area of toxicogenomics, proteomics or metabolomics that may give insight in a common mode of action. Targeted *in vitro* testing followed by selected *in vivo* tests can supplement the hypothesis testing. This research is thought to contribute to hypothesis based tiered testing approaches using existing toxicological data and structural similarities to chemicals with a known mode of action to formulate and refine a hypothesis on a possible mode of action of the target chemical. It is expected that future

[8] http://www.epa.gov/ncct/toxcast/

research in this area will promote a transfer from standard test batteries to hypothesis driven toxicity testing and yield more information on the mode of action and enable refined risk assessments (see also top of section 3.2.2.1).

These advancements may facilitate targeting testing to identify the pivotal effect, mechanism, and dose of interest, minimising animal use and reducing risk assessment uncertainty. The ToxCast™ program is expected to be applied to prediction and prioritisation of unknowns by 2012 (ToxCast Summit, 2009).

4.3.3 Critical body burdens in environmental risk assessment

A number of reviews have been made on the concept of Critical Body Burden (CBB), or Critical Body Residue (CBR), or Tissue Residue Approach (TRA) approaches (e.g. Barron *et al*, 1997, 2002; Sijm and Hermens, 2000; Thompson and Stewart, 2003). These approaches were also the subject of a SETAC Pellston workshop in 2007 which resulted in six review articles being published in Integrated Environmental Assessment and Management (see Meador *et al*, 2011 and subsequent articles). CBB approaches have been proposed as a more appropriate indicator of toxicity than the use of external media concentrations as they represent a more toxicologically relevant expression of dose (McCarty and Mackay, 1993). This can be explained by internal body concentrations, or body burdens, accounting for all routes of exposure rather than exposure via the environmental media alone. For highly hydrophobic substances, including those categorised as PBT/vPvB or POP, exposure via the water will be a much less relevant route of exposure than via the diet. Expressing toxicity as a measure of environmental concentration is the surrounding media is much less relevant, therefore, than the concentration within the internal target organs, or tissues.

The advantages of CBB approaches as outlined by McCarty and Mackay (1993) include:

1. Bioavailability is explicitly considered as uptake of a bioavailable fraction that would have to have occurred in order to be present within target organs.
2. Uptake kinetics can be considered a limitation of traditional toxicity tests when applied to highly hydrophobic substances. For substances such as these, uptake via the media may occur at such a slow rate that steady state concentrations within the tissues may not be achieved within the duration of traditional toxicity tests (Hendriks *et al*, 2001). CBB approaches have the benefit of being able to identify when steady state concentrations have been met.
3. Uptake from food is explicitly considered and with this being the more significant route of exposure for highly hydrophobic substances, dietary exposure will ensure that steady state concentrations are achieved sooner than via the media.

4. Toxicity (whether it be acute lethality or chronic sub-lethal effects) is expressed in a more meaningful parameter, particularly when used within a food chain assessment, as proposed by ECETOC (2005a). In such an assessment the internal body concentrations within food chain organisms, as caused by exposure through the diet, can be compared to the CBB known to cause a lethal or sub-lethal effect.
5. Influence of metabolism is considered as the internal body concentration that would encompass the ability of the organism to metabolise the substance.

Despite the benefits of applying CBB-based approaches within the risk assessment of substances such as PBT/vPvB and POPs, to date they have not been widely used or accepted over more traditional methodologies. This lack of adoption may be explained by the relative lack of residue-based toxicity data due to the need to develop standardised CBB-based toxicity testing protocols that form a part of an appropriate testing strategy. These limitations are currently being addressed in a CEFIC LRi funded research project titled "Critical body residue validation for aquatic organisms exposed to chemicals causing toxicity by baseline narcosis" (www.cefic-lri.org).

However, despite the lack of adoption of these methods within risk assessment, the US EPA (2008) have proposed the use of CBB-based approaches within the environmental risk assessment of pesticides with PBT characteristics, including the illustration of the method within a case study. Sappington *et al* (2011), at the 2007 Pellston workshop reviewed the use of TRA approaches within risk assessment. A conceptual framework for the application of TRA was proposed, methods for incorporating the TRA in risk assessment were discussed along with examples. Sappington *et al* (2011) conclude that:

- TRA and traditional media-based assessments of toxicity should be viewed as complementary rather than competing. Both approaches have strengths and weaknesses and the balance of these should be considered on a case-by-case basis.
- A tiered approach to the application of TRA can be adopted based on implicit, calculated and measured tissue residue response relationships.
- Despite the advantages of TRA, particularly for bioaccumulative chemicals, TRA should not be considered a panacea and must be applied with a clear understanding of underlying assumptions and limitations.

Progress is being made in the application of CBB approaches, but it should be noted that methods are considered more established for chemicals with a narcotic mode of action (Sappington *et al*, 2011, McCarty and Mackay, 1993; McCarty, 1986).

4.3.4 Biomonitoring equivalents (BE) in human health assessment

In 2008 a working group of regulatory toxicologists developed the concept of biomonitoring equivalents as a screening tool to interpret biomonitoring data in a public health risk assessment paradigm (Meek *et al*, 2008; Hays *et al*, 2008a,b, LaKind *et al*, 2008). Biomonitoring equivalents (BE) are defined as the concentration of a chemical (or metabolite) in a biological medium (blood, urine, human milk, etc) consistent with defined exposure guidance values, including reference doses and reference concentrations (RfD and RfC), minimal risk levels (MRL), or tolerable daily intakes (TDI) (Hays *et al*, 2008b). Today, most reference doses (safe levels for humans) are derived as external doses or concentrations in environmental media. The BE approach essentially tries to translate those external reference doses into internal dose levels in order to enable comparison with relevant biomonitoring results. The BE approach uses existing toxicological and pharmacokinetic information to derive internal dose levels of a chemical or the relevant metabolites that are thought to be the toxic species in blood or ideally at the target organ. These dose levels can then be compared to relevant concentrations derived from biomonitoring data of the relevant chemical or metabolite. Depending on the amount of information available for the derivation of the BE with regard to long term studies, pharmacokinetic information in animals and humans, knowledge on the mode of action and the molecular species responsible for the critical toxicological effect, the uncertainties in the BE will vary and need to be characterised on a case by case basis. The BE is at present viewed as a screening value that can be refined as more data become available. The point of departure for the derivation of a BE is typically a NOAEL, LOAEL or benchmark dose (corresponding to an estimated low effect level between 1 and 10%) of the most relevant animal study or human information. If additionally animal toxicokinetic data are available they are used to derive an animal internal dose. Then the usual inter and intra-species extrapolations and assessment factors are used to derive a human biomarker concentration that should not have an adverse effect in humans and would not trigger risk management actions. Similarly if only human toxicokinetic data are available one would apply the respective extrapolation and assessment factors on the animal study results and translate them thereafter into an internal human bioequivalent value using the human toxicokinetic information (Hays *et al*, 2008a,b; LaKind *et al*, 2008). The application of this approach to the risk assessment of PBT or vPvB chemicals is particularly of interest as those chemicals tend to be eliminated slowly. As outlined by Hays *et al* (2008b), when the rate of elimination is relatively slow compared to the frequency of exposure and the chemical has a long biological half-life, the chemical is likely to build up to approximate steady state levels and fluctuations in exposure have little impact on the measured biomarker concentration. Thus where the toxicological data used to derive a BE are likely to reflect a steady state situation for a considerable period of the respective study (normally after 2 to 3 half-lives) the level of uncertainty around the BE is relatively low. In fact at or near the steady state several dose metrics, as area under the curve, maximum, average and minimum concentrations give very similar results (Hays *et al*, 2008b).

In conclusion possible refinements of the database for chemicals with PBT or vPvB properties, include toxicological studies of sufficient duration to cover a steady state situation, robust toxicokinetic data and information on the mode of action and the appropriate biomarker of exposure. With such information, bioequivalent concentrations can be established and compared with high quality biomonitoring results of the respective biomarker in a refined risk assessment approach for human health. This can also be the basis for informed decisions on risk management.

4.4 Conclusions

The previous report (ECETOC, 2005a) developed an environmental risk assessment strategy for PBT chemicals which followed the general guidance described within the TGD, modified to reduce the uncertainties involved. This approach starts with a refined assessment at a higher tier. It takes advantage of additional data that would be available from confirming the PBT status of the chemical and any available environmental monitoring data or modelled data in more remote geographical regions. In addition, it includes evaluation of secondary poisoning and an effects assessment based on chronic effects, as well as using parameters such as MOA and CBBs to better assess endpoints of concern.

In addition to what was identified in the previous report (ECETOC, 2005a), there are more recently refined methodologies that can influence both *exposure* and *effects* assessments which are worthy of consideration in a risk assessment strategy for PBT chemicals. There is no defined scheme as each substance will have its own peculiarities and there will be a need to look at each chemical on a case-by-case basis and use more targeted, higher tier and invariably more expensive approaches in order to reduce the uncertainty of the risk assessment.

On the *exposure* side, multimedia modelling can have a much more significant role as a higher tier tool in PBT assessment, e.g. to prioritise the key environmental compartment and confirm where the weight of experimental evidence lies. Recent activities have focused on developing tiered or intelligent approaches to single media persistence assessment. New methods being developed should lead to the generation of more appropriate biodegradation kinetics that can distinguish between rates of removal due to sorption, partitioning and degradation. The use of models for exposure assessments provides greatest confidence when combined with monitoring data. The most reliable way of confirming exposure is to analyse the product in relevant environmental media. Such monitoring (e.g. VMSs) can confirm outputs from models, confirm persistence or degradation and be used to refine PECs used in the risk assessment. In particular where biomonitoring is carried out on populations over time, such time trends are helpful in assessing the response times of internal exposures after the introduction of exposure controls. Refinements for assessing bioaccumulation include the application of various validated models,

use of TMF or FWMF for quantifying biomagnification through the food chain or adoption of probabilistic risk assessment methodologies to assess TDI versus adverse effects.

An environmental *effects* assessment should specifically focus on longer-term tests in the environmental compartments of concern and provide a more refined food chain / secondary organism effects assessment than would be the case for non-PBT chemicals. A protein target MOA approach has been identified as having real potential in aiding the selection and design of *in vivo* aquatic toxicity tests. There is also interest in applying a comparative plasma concentration model which could be applied to provide read-across of pharmaco- and toxico-dynamic information on drugs from humans to fish. By identifying the MOA using targeted studies it may also be possible to identify early markers of substance-related changes for use in a refined risk assessment approach. Translation of *in vitro* technologies, developed during drug discovery to chemical assessments, has been based on both empirical (using HTS bioassays) and mechanistic (using 'omics tools and *in silico* methods) approaches. CBB-based approaches represent a more toxicologically relevant expression of dose for PBT chemicals and are expected in future to be adopted more widely once standardised CBB-based toxicity test protocols are developed. The application of biomonitoring equivalents is particularly relevant to the risk assessment of PBT chemicals which are eliminated slowly from the body and therefore tend to build up to steady-state levels. Biomonitoring data for a biomarker can then be used for a risk-based assessment.

5. RECENT DEVELOPMENTS AND FURTHER RESEARCH

A number of the areas where there is a need to improve the scientific knowledge upon which risk assessments are based have been progressed in recent years as the need for more refined risk assessments has developed. The chemical industry has endeavoured to contribute to increasing this knowledge base via a series of task forces, workshops and research programmes. In the first part of this chapter a number of areas are described where research, specifically targeted at developing the understanding of the behaviour and effects of chemicals to contribute to improving the risk assessment of chemicals (particularly chemicals meeting the PBT and POPs criteria), is either underway or is planned. The second part of the chapter identifies the areas where additional research would help to further increase the accuracy of the risk assessment and reduce uncertainties.

5.1 Persistence

The limitations of current testing for biodegradability and the necessity for improved laboratory tests have been widely recognised. During the preparation of the technical guidance documents for REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) the REACH Implementation Project (RIP 3.3) endpoint working group on degradation identified a number of modifications and enhancements to the current suite of biodegradation tests which could lead to an increase in the environmental relevance and predictability of biodegradation and environmental persistence.

There was broad consensus at the ECETOC workshop held in 2007 (ECETOC, 2007a) that a tier of enhanced biodegradation screening studies are required to aid in the prioritisation of PBT and vP/vB assessments. As a first step in the development of improved biodegradation tests, a list of reference chemicals covering a range of environmental persistence and non-persistence has been developed under LRi project ECO 12 under the guidance of an advisory committee comprised of regulatory body, academic and industry scientists (report is available from CEFIC LRi). This reference set will be available for use to validate modifications to existing biodegradation test methods and to develop new test methods. The validation set should also help address concerns that some of the modifications or new methods could result in tests becoming too powerful or overly protective. The aim of the research is to establish such a list of chemicals, with an agreed (by regulators and industry) set of properties and characterised set of degradation behaviour. The reference set of chemicals will be relevant to projects addressing:

- Compartment persistence / biotransformation.
- Determination of test method variability, e.g. via ring testing.
- Field monitoring/exposure model validation.

Many of the higher tiered fate studies have not been validated (ring-tested) and have no validity criteria or positive controls to allow effective benchmarking. Where studies have been validated and developed they have been done so on easily degradable chemicals. Work is needed to develop test methods, with and without adaptation, on non-readily biodegradable chemicals. In order to improve the scientific basis for persistency and biodegradation assessments it will be essential to consider the design, application domain and limitations of existing biodegradability tests. Enhancements discussed included extending the test duration, increasing the test volume, enhancing the biomass and allowing for acclimation. Whilst extension of the test duration and conducting studies using higher test volumes posed little concern to regulatory members of the workshop, it was felt that some validation and standardisation was needed with respect to working with increased biomass density and diversity and acclimated *inocula*. The work of Thouand *et al* (1995) action on the impact of *inoculum* density and diversity of biodegradation potential demonstrated that the probability of biodegradation increased at higher biomass concentrations due to the greater likelihood that competent degraders would be present. Understanding the relationship between biomass density and diversity for a range of *inoculum* sources may be a useful approach that should increase environmental realism, reduce the variability within and between tests, and increase the predictive nature of the tests. Consequently, research addressing the impact of diversity / density, how these might be considered in screening tests and finally the development of a low cost enhanced biodegradation screening test is being carried out under CEFIC LRi project ECO 11. The proposed new test will be validated using the reference chemicals for persistence described above (ECO 12) and the final protocol will be submitted to the OECD or ISO test guidelines programme. This project is considered to be fundamental to the discussions about how to test chemicals for their biodegradation in the environment and how to generate the values for half-lives needed for PBT and risk assessment. It is envisaged that such enhanced tests will contribute to a weight of evidence approach to decide if a chemical is persistent.

ECETOC (2009) reviewed the default rate constants assigned to ready and inherent biodegradation tests to determine predicted environmental concentrations in marine and freshwater habitats. This review concluded that there was a lack of data to judge the scientific basis of the 150-day half-life assigned to inherently biodegradable chemicals. Consequently, research is required to assess the rates of biodegradation of non-readily biodegradable chemicals in marine and freshwater degradation studies to determine relevant residence times and appropriate environmental half-lives.

Higher tiered studies such as the OECD Transformation in aerobic and anaerobic water sediment system (OECD TG 308, 1994) were developed in an attempt to develop a simulation of environmental conditions. This test is often used as a surrogate for assessing degradation in rivers. This is a false assumption as it was originally designed to assess the fate of pesticides that enter shallow ditches as a result of spray drift. The artificially high level of sediment relative to

water (1:3) renders the test as unsuitable for assessing the fate of down-the-drain chemicals that enter environmental waters such as rivers and lakes. Having a two-phase system, with water and sediment, makes the test very difficult to interpret with DT50 values combining rates of dissipation and degradation. Contrary to the screening level tests (OECD 301 and 302 series), the higher tiered tests (e.g. OECD 308 and 309) have not been tested extensively validated through ring-testing and the OECD 308 also lacks any quality assurance or quality control measures (e.g. validity criteria or positive or negative controls). Research is required to (i) develop appropriate higher tiered screening studies with suitable quality control and quality assurance data and (ii) validate the use and relevance of existing higher tiered degradation tests for exposure and persistence assessment with appropriate test chemicals.

5.2 Bioaccumulation

5.2.1 Biotransformation

Information on biotransformation and biodegradation can be particularly important in:

- PBT assessments, where a weight of evidence approach can be used to support decisions made on the potential for chemicals to bioaccumulate or to degrade in the environment. Understanding relationships between degradation in microbial systems and biotransformation and bioconcentration in higher trophic levels (e.g. invertebrates, vertebrates) will help in such a weight of evidence approach.
- Aquatic, terrestrial and marine assessments, where a technical basis is needed for the extrapolation of biotransformation rates of chemicals between vertebrate species (e.g. fish, rats, humans) which can thus be incorporated into food-chain models.

Bioaccumulation is defined as the net result of uptake, distribution, metabolism and excretion of a substance in an organism and was the subject of a review by ECETOC in 1995. The potential of a substance to bioaccumulate in the aquatic environment into an organism depends particularly on its lipophilicity and its metabolism within the organism (Feijtel *et al*, 1997). When aquatic organisms are exposed to chemicals in the environment, some of these chemicals maybe universally metabolised, whilst others are either metabolised by specific species / taxa or are non-metabolisable and therefore may bioaccumulate. An LRi sponsored project has been completed in which quality assessed data on the metabolic pathways of chemicals in aquatic organisms was reviewed, collated and then analysed using regular expression pattern matching technology to identify structural fragments that are susceptible to biotransformation. These data have been encoded into a software application called ‘BiotS’ (Biotransformation Susceptibility). The study dataset contained data on 85 biotransformation pathways involving 342 chemicals and 316 metabolic steps in 58 aquatic species (http://www.ecochemistry.co.uk/downloads/).

A project which builds upon this initial work and aims to establish relationships of biotransformation across organisms has now been funded by CEFIC LRi. Based on a critical review of available literature, quantitative relationships relating to biodegradation, biotransformation and bioaccumulation in organisms from different taxa and trophic levels, particularly with respect to parent compound biotransformation rates will be analysed and developed. Of particular interest is the development of relationships between microbial system, invertebrates and vertebrate species (e.g. fish, mammals, humans). Chemical structures associated with universal lability, limited or specific lability, and no lability (or practically none) will be highlighted. Links will be developed with other researchers to support the development and validation of *in silico* and *in vitro* biotransformation models and/or assays. Published data will be critically reviewed and collaboration with other groups (e.g. ILSI-HESI) will be established to support the development of *in vitro* assays for estimating biotransformation rates in key species for the regulatory assessment of chemicals, including fish and mammals. Information will be provided to support the validation of promising *in vitro* systems, e.g. trout and carp S9, zebrafish embryos and hepatocytes, that measure biotransformation in collaboration with other parties (e.g. ECVAM).

A February 2011 ILSI-HESI workshop on "Moving Bioaccumulation Assessments to the Next Level: Progress Made and Challenges Ahead" summarised recent findings, prioritised research needs, and identified regulatory issues not being addressed by ongoing or planned work. Common needs identified across all topic areas included improved and open data access, development of benchmarks for methods and processes, determination of the domain of application for each method, addressing uncertainty in 'B' assessments, and increased dialog with the regulatory community as a means of promoting acceptance of new data and methods. The full workshop report is available at:
http://www.hesiglobal.org/i4a/pages/index.cfm?pageid=3568. The following section provides an overview of the discussions related to research on *in vitro* biotransformation discussed at the workshop. Though bioaccumulation of a chemical is the result of absorption, distribution, metabolism and excretion (ADME) processes, most models neglect the contribution of metabolism as a clearance mechanism, potentially leading to inaccurate estimates of bioaccumulation potential. In addition, no models are currently available which are capable of accurately estimating potential rates of metabolism. Though the *in vivo* OECD 305 fish bioconcentration factor (BCF) test (OECD, 1996) accounts for metabolism, it requires large numbers of test organisms and is time- and cost-intensive. There is a need to close this gap between *in silico* and *in vivo* approaches with an efficient *in vitro* testing strategy that includes not only an assessment of passive uptake but also biotransformation and proper scaling from test tube to whole animal.

Incorporating xenobiotic metabolism by Phase I and II metabolising enzymes in fish is critical to improve bioaccumulation estimates (Smítková *et al*, 2005; de Wolf *et al*, 2007). These *in vitro*

metabolism data can be incorporated into BCF prediction models and to extrapolate *in vitro* test results to whole body biotransformation rates (k_{met}) to refine BCF model predictions (Cowan-Ellsberry *et al*, 2008).

Due to the availability of commercial cryopreserved fish S9 fractions and the ease of conducting *in vitro* biotransformation testing with these fractions, an extensive pre-validation study using rainbow trout S9 fractions was coordinated by ILSI-HESI with funding provided by CEFIC-LRi and ECVAM. This effort led to the development of a standardised protocol and evaluation of nine test compounds in five different laboratories. However, continued evaluation of these 'optimised' methods is necessary, in addition to increased focus on key issues such as observed high inter-laboratory variability and its impact on predicting 'B'.

Additional work is necessary to resolve and explain inter-laboratory variability in these S9 study results, including the impact of this variability on overall bioaccumulation estimates, as well as further development of *in vitro* to *in vivo* extrapolation models by collecting additional physiology and binding parameter data. In order to gain regulatory acceptance and to propagate trust in these *in vitro* methods, test relevance must be shown by demonstrating biological relevance and extrapolation of *in vitro* results to *in vivo* BCF test results when possible. This will involve close interaction with researchers developing new modelling tools as well as those developing new *in vivo* methods.

5.2.2 Food webs

The importance of the role of biotransformation in the modelling of bioaccumulation and biomagnification in food webs for different environmental compartments was highlighted in two projects funded by CEFIC LRi. IFREMER (2003) developed a food web model as part of a programme to describe the fate of organic chemical contaminants in estuarine food webs (GEMCO). Two simplified food webs were developed, one typical of round fish the other one of the flat fish, both of which are representative of main food chains in estuarine ecosystems. The model, applicable for neutral organic substances but not for metals or ionised organic substances, estimates the propensity for a compound to bioaccumulate or to be biotransformed in estuarine food webs. The model was validated for PCBs, (log K_{ow} in the range 5-7) in the Baie de Seine and in the Seine estuary. An approach was proposed to account for biotransformation, based on experimental results for benzo(a)pyrene (BaP) which showed rapid biotransformation. The BaP data were used to define an empirical biotransformation factor. The concentration of a less persistent compound or partially metabolisable compound is calculated by the PCB bioaccumulation model and then corrected by a biotransformation factor.

In the second project, Alonso *et al* (2008) reviewed the kinetic models covering species that belonged to different trophic levels and with different ecological behaviour and concluded that a

tiered conceptual biomagnifications model could be developed starting with a simplified food chain which could then be refined to produce more realistic and complex models in successive tiers. A simplified but versatile model was proposed to estimate biomagnification potentials in a generic food web. The model is based on single-compartment kinetics for each organism within a trophic chain. Exposure patterns and main biological events (reproduction, starvation, etc.) can also be defined for the defined time period and therefore, if the time units are days, daily fluctuations can be predicted in exposure and chemical releases due to reproduction. For the marine environment the experimental design was based on exposure from food. The selected species were the unicellular algae (Isochrysis galbana), the filtering bivalve (Mytilus edulis) and the fish (Sparus aurata). Various compounds were studied, exhibiting various physico-chemical properties, structural differences, and different environmental behaviour. Organisms were exposed to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153); 3,4,3',4'-tetrachlorobiphenyl (CB-77); 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153) (which are considered to be very slowly metabolised, if at all), or to PAHs (chrysene, benzo(a)pyrene, fluoranthene) which are metabolised in fish, to investigate the importance of biotransformation in the model predictions.

5.2.3 Trophic magnification factor (TMF)

Environmental exposure of aquatic and sediment organisms to lipophilic compounds may occur through the water column, diet, and/or sediment. At a recent SETAC Pellston workshop (2008) on bioaccumulation processes, primarily in aquatic systems, it was proposed that bioaccumulative substances be defined as "substances which biomagnify in the food web" – i.e. lipid-normalised concentrations increase with increasing trophic position (SETAC, 2008). Based on this definition, it was concluded that the most relevant criterion for assessing the potential of a chemical to bioaccumulate was the trophic magnification factor (TMF), and that the most conclusive evidence to demonstrate that a chemical substance biomagnifies, was a TMF > 1. TMFs are increasingly being used to assess bioaccumulation and biomagnification of chemicals in the environment (Houde *et al*, 2008) and data suggest that TMFs may be broadly applied across systems that differ considerably in their location and characteristics.

The TMF (see section 4.2.4.2) is considered to be a valuable parameter for the sound assessment of a compound's potential to bioaccumulate / biomagnify in aquatic food webs. A general hierarchical ranking of bioaccumulation measures for highly lipophilic chemicals (log K_{ow} >5), particularly in aquatic systems, has been proposed (Gobas *et al*, 2008): (1) TMF, (2) biomagnification factors (BMF), and (3) bioconcentration factors (BCF). A CEFIC LRi funded project is currently studying the relationships between BCF, BAF, BMF and TMF (LRi ECO 9). The main objectives of the study are:

- To understand the important factors impacting BCF and BAF measurements.

- To study the links between BCF, BAF, BMF and TMF.

An empirical and theoretical approach is being used. Bioaccumulation in the field is being studied in the Western Scheldt estuary which was selected as the field site because it has large land-based pollution inputs and there are many different chemicals present at concentration significantly above the detection limits for the different compartments of the ecosystem. The theoretical approach involved the use of the Gobas and Arnot (2010) food-web bioaccumulation model which has been parameterised and calibrated to represent the Western Scheldt food web. The calibrated model is being used to explore the relationships between BCF, BAF and the TMF, identify the mechanistic factors that control the relationship between the BCF, BAF and TMF and itions under which the BCF based bioaccumulation criteria can be used to identify bioaccumulative substances. The results demonstrate the importance of including bioavailability in the water phase on the calculations of bioaccumulation metrics such as BAF as well as the impact of biotransformation on the biomagnifications (BMF) and trophic magnification factors (TMF).

BCF and BAF, therefore, do not always correlate with BMF and TMF demonstrating that the bioaccumulation assessment of chemicals should be based on various bioaccumulation metrics.

The importance of biotransformation as important factor impacting the bioaccumulation of chemicals in a food chain was examined in relation to the BCF, BAF, BMF and TMFs.

It was concluded that:

- BCF values are clearly subject to variability (due to protocols followed, but also species-specific differences).
- The use of dissolved concentrations has an impact on the BAF calculated in the field, and highlights the influence of bioavailability on bioaccumulation.
- BCF is often higher than the BAF if the BAF is calculated on total water concentrations.
- When the BAF is examined, it can be seen that for some compounds, such as Perfluorooctanoic acid (PFOA), the median BAF value is very low (79); however the TMF is > 1 in the benthic food web, suggesting biomagnification.
- The results for pyrene and decabromodiphenyl ether (BDE 209) show that biotransformation can have a major impact on bioconcentration and field bioaccumulation. This also contributes to a low TMF: Trophic dilution.
- Any reliable B-assessment of a chemical should be based on a variety of bioaccumulation metrics.

Current assessment methods to determine a chemical's TMF involve extensive field sampling and analytical residue analysis of a large number of food web organisms across several trophic levels, a process which is expensive, time-consuming, and animal intensive (e.g. Houde *et al*, 2008). For

new chemicals or those that are not widely found in the environment due to limited environmental releases, alternative methods must be developed for assessment of TMF. A range of tools are known to exist, such as solid-phase microextraction (SPME) methods for rapid measurement of residue concentrations (lipid-adjusted), a variety of *in silico* and *in vitro* methods to determine metabolism, and computer models that purport to predict biota concentrations in aquatic species of a constructed food web (e.g. AQUAWEB, ACC-Human, and TAO). Such methods may be used together or with other approaches to produce an estimation of a chemical's TMF. The need to develop a technique for the rapid estimation of TMF using laboratory, field and computer modelling methods in aquatic organisms has been recognised and is now being addressed under CEFIC LRi funding.

5.3 Ecotoxicity

5.3.1 Critical body burden

Over the past years several projects have focussed on exposure assessment of POPs/PBTs and have considerably improved the tools and knowledge. In particular modelling tools have been developed and improved and are now in the phase that they can be used reliably for certain classes of compounds, in particular narcotic chemicals. In parallel to human biomonitoring there is also an urgent need to improve the possibilities to interpret measured environmental exposure data in a toxicological context. For POPs/PBTs a promising development is the use of CBB, CBR or critical body concentrations, in particular for chronic effects. This approach has two distinct advantages. Firstly it significantly improves the uncertainty of the effect assessment, as compared to using ‘classical' toxicity data. Secondly, exposure data on POPs/PBTs often come from (bio)monitoring; for example as mg/kg body weight measurements, and CBBs directly provide a context for interpretation of such data. CBB data are available, but these are neither readily available nor properly validated; they are limited and in most cases restricted to acute toxicity. The objective of LRi project ECO 16 is to generate a validated CBB database and validate a CBB chronic toxicity range for narcotics.

The researchers will collate and validate the available data, preferably in a group approach for narcotics (or other MOAs) so that the number of data can be significantly increased. Currently available LBB (lethal body burden)/CBB data, starting from the LETR database (Jarvinen and Ankley, 1999), will be supplemented with additional data resulting from a review of recent scientific literature search and a user-friendly CBB database will be prepared. A validation system for LBB/CBB data will be developed and applied to the database. In the final phase a testing programme will be designed and executed to validate the CBB toxicity range for narcotics (2-8 mmol/kg). Several publications have indicated that the CBB of narcotic chemicals is in the range of 2-8 mmol/kg. In practice this is sometimes used but still debated. The current project intends to generate sufficient confidence to allow broad use. CBB data can directly be used in

risk assessments of PBTs and in interpreting monitoring data, especially data from biota and predators high in the food chain.

5.3.2 Bound residues and sediment toxicity testing

There are several internationally agreed tests available for investigating the potential long-term effects of substances, metabolites (or transformation products) and non-extractable residues on sediment-dwelling organisms. These standardised test methods focus on benthic invertebrates (e.g. *Chironomus, Lumbriculus*). However, there is a lack of methods for assessing potential effects on microorganisms, plants and animals from different taxonomic groups. The European Commission guidance for REACH (Registration, Evaluation, Authorisation and restriction of Chemicals; ECHA, 2008b) identifies that specific effects of chemicals on plants and microorganisms are not covered by the available test methods. The guidance also states "as these organisms also play an important role in the benthic community, there is the necessity to further develop standard test methods and to revise the sediment assessment concept accordingly in the future".

Development of acute and chronic sediment tests covering different trophic levels and exposure pathways are needed to better define the potential environmental risks posed by chemicals in sediments. In selecting key species for assessing toxicity it is necessary to also consider exposure pathways (e.g. via pore water or ingesting sediment particles). Indeed, the exposure route is strongly influenced by species-specific feeding mechanisms and the behaviour of the organisms in, or on, the sediment (e.g. surface deposit versus burrowing sub-surface feeders). Such factors influence toxicity and the outcome of test results. However, scientific knowledge and guidance are also lacking on the selection of appropriate test species and test designs.

Consideration of toxicity data from a wider range of species / exposure routes can be expected to help improve guidance on species selection and increase confidence in deriving no-effect concentrations for sediment communities. CEFIC LRi project ECO 17 will develop guidance to assist regulators in selecting ecologically relevant endpoints to reflect the appropriate routes of exposure**.** A risk assessment framework for prospective testing will be developed and guidance given on how sediment ecotoxicity data should be used within the context of Environmental risk assessment (ERA) to support management decisions.

5.4 Monitoring and Modelling

5.4.1 Monitoring data

The highest tier of refinement for an exposure assessment is the use of measured concentration data. A project was initiated in 2004 to facilitate the retrieval of background concentrations and ranges of organic chemical concentrations in the marine and freshwater environments as input to various multimedia models. The product of this research was a database (MonitoringBase, freely available on CD from CEFIC, Brussels). The database includes information, references and links to more than 160 planned, ongoing and completed contaminant monitoring studies in the European and Arctic aquatic environments. The information and references can be used to optimise cooperation with ongoing and planned monitoring campaigns which will act to maximise the data and information and minimise the number of samples collected.

5.4.2 Modelling and QSAR

Model and QSAR approaches can help to refine risk assessments of PBT/vPvB chemicals and provide tools for prioritisation and benchmarking of different chemicals. Refinement of the risk assessment may in some cases confirm a risk to the environment or human health while in other cases it may demonstrate the absence of risks with an acceptable level of uncertainty.

One of the key concerns proposed to be addressed through the PBT scheme is the potential for long distance exposure. Just as population models can be fine-tuned for specificity to address small ecosystems, they can also be generalised to predict regional, broad range exposure scenarios. However, research into spatial behaviour of chemicals and populations and their interactions is needed to direct testing of potentially-exposed species. This is one of several aspects of modelling being explored by CREAM, a network funded by the European Commission under the 7th Framework Program (http://cream-itn.eu/). The overall aim of the network is the development of "Mechanistic Effect Models for Ecological Risk Assessment of Chemicals" one aspect of which is population modelling. To make informed, pragmatic decisions about the consequences of the long-term use of chemicals a better understanding of population and ecological dynamics and the extrapolation of responses from single-species, single endpoint laboratory studies to diverse populations in varying exposure scenarios is needed. Similar needs are being addressed in areas of ecological management such as conservation biology through population modelling. (Forbes *et al*, 2008).

It is hoped that the work of this group and others like it, including the recently formed SETAC – Europe Advisory Group; "Mechanistic effect models for ecological risk assessment of chemicals (MEMoRisk)" (Preuss, 2009) can bring environmental risk assessment beyond the conservative assessment factor approach and to place where PBT concerns can be scientifically addressed.

5.5 Further areas for research

Whilst some work is in progress to improve the scientific basis for the assessment of persistence there are still a number of other areas that may need to be addressed if more accurate and realistic rates of biodegradation are to be measured for chemicals which are predicted to meet the P criteria.

Biodegradation influences exposure and hence it is a key parameter in the estimation of the risk of long-term adverse effects on biota. Biodegradation rates are determined in, or assigned from, the results of laboratory based degradation tests. How such biodegradability data should be interpreted and analysed, especially data from higher tier biodegradability tests, is an issue that needs to be addressed. A large number of system specific and microbiological factors are involved in the biodegradation of a given chemical in the various environmental compartments. Consequently the use of one half-life or rate constant as the all encompassing number to characterise the behaviour of a chemical is fundamentally flawed. Rather than giving actual numerical estimates of half-lives these should be assigned to a range with the size of the range varied according to the perceived accuracy of the determination (i.e. the half-life bin concept). By measuring half-lives from a 'battery' of tests, half-life distributions could be generated. These could be compared to single values and assessed for their usefulness within the regulatory decision making process.

There is a growing consensus that there are no biodegradation studies that accurately simulate biodegradation in the 'natural' environment. Higher tiered biodegradation studies (e.g. OECD 307, 308 and 309) use environmental media that can describe degradation under conditions that have a greater environmental relevance than the ready biodegradation test. However, many of these studies do pose considerable problems associated with their interpretation. Problems identified included:

- Selection of the most appropriate compartment for testing.
- How to correct for temperature differences and is it really necessary?
- Cometabolism.
- How to deal with bound residues?.
- How to discriminate between degradation half-lives for persistency assessments and rates of dissipation from water or sediment?

Assessing biodegradation can be further complicated by the fact that a number of chemicals will only be degraded in the presence of other carbon and energy sources (i.e. are cometabolised) and may not be linked to microbial growth. The issue of cometabolism is particularly important when studies are performed at very low (ng/L) substance concentrations and is

particularly relevant in seawater environments where additional sources of carbon and energy are present in the real world.

The issue of metabolites and bound residues, including non-extractable residues, is an important factor in PBT assessment and risk assessment of chemicals. Precautionary risk assessments usually assume 100% bioavailability i.e. all of the chemical present is available, for degradation or to have potential toxic effects on the biota. This precautionary approach generally overestimates the exposure concentration by the amount that is not available and therefore overestimates the level of risk to biota in the environment. Although it is a position that has been recognised and referenced by REACH (ECHA, 2008c) and OECD test guidance (2002a,b), there is no agreed guidance on how to determine what is available and what is not, and how information on bound residues should be interpreted in the risk assessment. As a result, it continues to be debated from a scientific and regulatory point of view. ECETOC held a workshop in 2009 to further this debate and develop guidance on how to account for bound residues and bioavailability in environmental risk assessment. The conclusions, future regulatory and research needs and a framework outlining a possible approach for advancing and improving the risk assessment of bound residues are described in ECETOC Workshop Report 17 (ECETOC, 2010a).

Further research could also address the question of how existing knowledge on human pharmacokinetic models can be used to extrapolate to other environmental species. The integration of prediction models for exposure-time relationships, estimates of time to steady state and steady state concentrations in relevant receptors with food-chain and toxicokinetic models could lead to a decrease of uncertainty in the prediction of time and response times and concentration developments in target compartments and organisms following emission reduction. For the development of further integrated models it is crucial to define e.g. via sensitivity analysis which input parameters need to be determined and refined in order to decrease uncertainties in the model predictions.

On the effects side a better identification of the modes of action and relevant NOAECs could be achieved through a combination of ‘omic-technologies, *in vitro* studies and targeted *in vivo* experiments.

Further research is also needed to scientifically derive cut-off values of concern for biomagnification and overall persistence.

One area of uncertainty in the risk assessment of PBT /vPvB chemicals is the emission estimates. A better characterisation of emissions with a combination of measured data and emission modelling based on a robust data set of measured data on defined processes is also an area for further research.

6. CONCLUSIONS

ECETOC (2005a) proposed a scheme for the risk assessment of PBT chemicals. This scheme was built using a number of higher tier risk assessment refinement approaches that were developed to reduce the uncertainties associated with exposure, effects and food chain assessments, and remains valid. The purpose of this report has been to further develop these proposals by identifying refinement options taken from experiences gained in the risk evaluation of PBT, vPvB and POPs, as well as scientific advancements made over the last five years.

From the example on risk based evaluations reviewed in Chapter 3 of this report it is clear that there can be no single defined scheme or framework that can, in simple terms, cover every eventuality of a high tier risk assessment for chemicals categorised as PBT or vPvB. Whilst there are a number of generic elements that should be included within such a risk assessment (e.g. high level of refinement in both exposure and effects assessment, the need for a food chain assessment, monitoring of environmental concentrations along both a spatial and temporal scale), the detail of the assessment strategy that would be required to adequately reduce the uncertainties associated with each chemical in question would need to be considered on a case-by-case basis. Despite similarities that can be drawn due to their persistent, bioaccumulative and toxic profile, each high tier risk assessment must take into account the specific properties and use scenarios for the chemical in question in order to address its specific concerns.

This bespoke (or tailored) high tier risk assessment strategy would benefit from the information that would need to be available in order to resolve any issues associated with the categorisation of the chemical as a PBT or vPvB. However, this information alone would not be sufficient in order to adequately assess the risks associated with the chemical and a significant amount of information would need to be generated, in a targeted manner, in order to sufficiently reduce the uncertainties. The generation of such information would require a significant investment of resources, possibly on an ongoing basis, as is appropriate for a substance of high concern.

Chapters 3 and 4 of this report have identified a number of refinement options which can be used in the high tier risk assessment of a substance categorised as PBT or vPvB. These refinement options include:

- Use of P_{ov} instead of compartment specific considerations of persistence.
- Advanced fate modelling tools to predict spatio-temporal variations in environmental concentrations.
- (Bio)monitoring to measure concentrations in relevant environmental compartments over a spatio-temporal scale, as well as to assess and improve the accuracy of environmental fate models.

- Use of probabilistic methods to better account for the uncertainties associated within an exposure or effects assessment.
- CBB (or TRA) approaches, accompanied by an understanding of Mode of Action.
- Use of experimentally derived BMF and TMF in food chain assessments.

The use of these, as well as other, high tier risk assessment refinement options must be targeted for use to address the specific concern of the substance in question. The use of chemical space mapping may be useful in this regard.

In addition, Chapter 5 identifies key research that is underway or planned which is aimed at reducing the uncertainties associated with chemical risk assessment. These areas of research further contribute to the refinement strategies required to risk assess chemicals which may be considered persistent, bioaccumulative and/or toxic.

ABBREVIATIONS

ADME	Absorption, distribution, metabolism and excretion
B	Bioaccumulative
BAF	Bioaccumulation factor
BaP	Benzo(a)pyrene
BCF	Bioconcentration factor
BE	Biomonitoring equivalents
BMF	Biomagnification factor
CAS	Chemical Abstracts Service
CBB	Critical body burden
CBR	Critical body residues
CEFIC	The European Chemical Industry Council
CEPA	Canadian Environmental Protection Act
CES	Centre européen des silicones
CTD	Characteristic travel distance
cVMS	Cyclic volatile methylsiloxane
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
D6	Dodecamethylcyclohexasiloxane
DBT	Dibutyltin
DDE	Diphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EAF	Exposure assessment factor
EC	European Commission
EC_{50}	Median effective concentration
ECB	(Former) European Chemicals Bureau
ECHA	European Chemicals Agency
ECOSAR	Ecological Structure Activity Relationships (computer program)
ECVAM	European Centre for the Validation of Alternative Methods
EDI	Estimated daily intake
EEC	Estimated environmental concentration
EMA	European Medicines Agency
ER	Effect ratio
ESR	Existing substances regulation
ETNC	Environmental threshold of no concern
EU	European Union
EUSES	European unified system for the evaluation of substances

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Feeding rate
FSSPC	Predicted steady state plasma concentration in fish
FWMF	Food web magnification factor
Gamma HCH	Gamma-hexachlorocyclohexane or lindane
GENEEC	Generic estimated environmental concentrations
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCBD	Hexachlorobutadiene
HL	Half-life
HSDB	Hazardous Substance Database
HTPC	Human therapeutic plasma concentration
HTS	High throughput screening
IC_{50}	Inhibiting Concentration 50%
IEAM	Integrated Environmental Assessment and Management
ILSI-HESI	International Life Sciences Inst. - Health and Environmental Sciences Institute
IPCS	International Programme for Chemical Safety
ISR	Interspecies sensitivity ratio
ITS	Intelligent testing strategies
LBB	Lethal body burden
LC	Lethal concentration
LD	Lethal dose
LIF	Swedish pharmaceutical trade association
LOAEL	Lowest observed adverse effect level
LOC	Level of comparison
LRi	Long-range Research initiative
LRT	Long-range transport
LRTP	Long-range transport potential
MOA	Mode of action
MOE	Margin of exposure
MPA	Swedish Medical Products Agency
MX	Musk xylene

NIR	Normalised intake rate
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
OECD	Organisation for Economic Co-operation and Development
OPP	Office of Pesticide Programs
OSPAR	Oslo Paris Convention
P	Persistent
PAH	Polycyclic aromatic hydrocarbon
PBDE-99	2,2′,4,4′,5-pentabromodiphenyl ether
PBPK	Physiologically-based pharmacokinetic (model)
PBT	Persistent, bioaccumulative, toxic
PBTK	Physiologically based toxicokinetic (model)
PCB	Polychlorinated biphenyls
PDFs	Probability density functions
PEC	Predicted environmental concentration
PFOA	Perfluorooctanoic acid
PMRA	Pest Management Regulatory Agency (Canada)
PNEC	Predicted no effect concentration
POP	Persistent organic pollutant
P_{ov}	Overall persistence
p,p'-DDE	p,p'-diphenyldichloroethylene
QSAR	Quantitative structure activity relationship
RAIDAR	Risk Assessment IDentification And Ranking
RAR	Risk assessment report
RCR	Risk characterisation ratio
REACH	Registration, evaluation, authorisation and restriction of chemicals
RED	Reregistration eligibility decision
RIVM	Netherlands National Institute for Public Health and the Environment
RQ	Risk quotient

SAR	Structure activity relationship
SCC	Stockholm County Council
SCCP	Short chain chlorinated paraffins
SETAC	Society of Environmental Toxicology and Chemistry
SPM	Suspended organic matter
STEP	Simulation testing of environmental persistence
STP	Solar-terrestrial physics
SVHC	Substance of very high concern
T	Toxic
TBB_U	Total body burden
TBT	Tributyltin
TBTCl	Tributyltin-chloride
TBTO	Tributyltin oxide
TBTOH	Tributyltin hydroxide
TC NES	Technical Committee of New and Existing Chemical Substances
TDI	Tolerable daily intake
TE	Travel efficiency
TGD	Technical guidance document
TL	Trophic level
TMF	Trophic magnification factor
TPT	Triphenyltin
TRA	Tissue residue approach
TSMP	Toxic Substances Management Policy (Canada)
UBA	Umweltbundesamt (German Environment Agency)
UF	Uncertainty factor
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
VMS	Volatile methyl siloxane
vPvB	Very persistent, very bioaccumulative
WHO	World Health Organisation
WOE	Weight of evidence

BIBLIOGRAPHY

Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L, Zenick H. 2006. The use of biomonitoring data in exposure and human health risk assessments. *Environ Health Perspect* 114(11):1755-1762.

Alonso E, Tapie N, Budzinski H, Leménach K, Peluhet L, Tarazona JV. 2008. A model for estimating the potential biomagnification of chemicals in a generic food web: Preliminary development. *Environ Sci Pollut Res Int* 15(1):31-40.

Angerer J, Ewers U, Wilhelm M. 2007. Human biomonitoring: State of the art. *Int J Hyg Environ Health* 210(3-4):201-228.

Arcus-Arth A, Krowech G, Zeise L. 2005. Breast milk and lipid intake distributions for assessing cumulative exposure and risk. *J Expo Anal Environ Epidemiol* 15(4):357-365.

Arnot JA, Mackay D. 2008. Policies for chemical hazard and risk priority setting: Can persistence, bioaccumulation, toxicity and quantity information be combined? *Environ Sci Technol* 42(13):4648-4654.

Arnot JA, McCarty LS, Armitage JM, Toose-Reid L, Wania F, Cousins IT. 2009. An evaluation of hexabromocyclododecane (HBCD) for persistent organic pollutant (POP) properties and the potential for adverse effects in the environment. [http://chm.pops.int/convention/POPsReviewCommittee/hrPOPRCMeetings/POPRC5/POPRC5documents/tabid/592/language/en-us/default.aspx]

Arnot JA, Armitage JM, McCarthy LS, Wania F, Cousins IT, Toose-Reid L. 2011. Toward a consistent evaluative framework for POP risk characterization. *Environ Sci Technol* 45(1):97-103.

Bailey RE. 2001. Global hexachlorobenzene emissions. *Chemosphere* 43(2):167-182.

Barber JL, Sweetman AJ, Jones KC. 2005a. Euro Chlor Science dossier: Hexachlorobenzene – Sources, environmental fate and risk characterization.

Barber JL, Sweetman AJ, van Wijk D, Jones KC. 2005b. Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *Sci Total Environ* 349(1-3):1-44.

Barron MG, Anderson MJ, Lipton J, Dixon DG. 1997. Evaluation of critical body residue QSARs for predicting organic chemical toxicity to aquatic organisms. *SAR QSAR Environ Res* 6(1-2):47-62.

Barron MG, Hansen JA, Lipton J. 2002. Association between contaminant tissue residues and effects in aquatic organisms. *Rev Environ Contam Toxicol* 173:1-37.

Beck J, Hansen KE. 1974. The degradation of quintozene, pentachlorobenzene, hexachlorobenzene and pentachloroaniline in soil. *Pestic Sci* 5(1):41-48.

Bennett DH, McKone TE, Matthies M, Kastenberg WE. 1998. General formulation of characteristic travel distance for semivolatile organic chemicals in a multimedia environment. *Environ Sci Technol* 32(24):4023-4030.

Beyer A, Mackay D, Matthies M, Wania F, Webster E. 2000. Assessing long-range transport potential of persistent organic pollutants. *Environ Sci Technol* 34(4):699-703.

Bezalel L, Hadar Y, Fu PP, Freeman JP, Cerniglia CE. 1996. Initial oxidation products in the metabolism of pyrene, anthracene, fluorene, and dibenzothiophene by the White Rot Fungus *Pleurotus ostreatus*. *Appl Environ Microbiol* 62(7):2554-2559.

Blunden SJ, Chapman A. 1986. Organotin compounds in the environment. In Craig PJ, ed, Organometallic compounds in the environment, chapter 3, Wiley, New York, NY, USA, pp 121-137.

Boethling R, Fenner K, Howard P, Klečka G, Madsen T, Snape JR, Whelan MJ. 2009. Environmental persistence of organic pollutants: Guidance for development and review of POP risk profiles. *Integr Environ Assess Manag* 5(4):539-556.

Brooke DN, Crookes MJ, Gray D, Robertson S. 2009. Environmental risk assessment report: Decamethylcyclopentasiloxane. Science Report SCHO0309BPQX-E-P. Environment Agency, Bristol, UK.

Callahan MA, Slimak MW, Gabel NW, May IP, Fowler CF, Freed JR, Jennings P, Durfee RL, Whitmore FC, Maestri B, Mabey WR, Holt BR, Gould C. 1979. Water-related environmental fate of 129 priority pollutants. Volume 1: Introduction and technical background, metals and inorganics, pesticides and PCBs. US EPA-440/4-79-029a. Final Report. pp. 27-1 to 27-16. US Environmental Protection Agency, Office of Water Planning and Standards, Washington, DC, USA.

CEFIC LRi ECO 9. Relationships between BCF, BMF and BAF: Improving forecasting of residues in biota in the environment based on laboratory testing. Research in progress.

Choudhry GG, van den Broecke JA, Hutzinger O. 1983. Formation of polychlorodibenzofurans (PCDFs) by the photolyses of polychlorobenzenes (PCBzs) in aqueous acetonitrile containing phenols. *Chemosphere* 12(4-5):487-492.

Cowan-Ellsberry CE, Dyer SD, Erhardt S, Bernhard MJ, Roe AL, Dowty ME, Weisbrod AV. 2008. Approach for extrapolating *in vitro* metabolism data to refine bioconcentration factor estimates. *Chemosphere* 70(10):1804-1817.

Cowan-Ellsberry CE, McLachlan MS, Arnot JA, MacLeod M, McKone TE, Wania F. 2009. Modeling exposure to persistent chemicals in hazard and risk assessment. *Integr Environ Assess Manag* 5(4):662-679.

Czub G, McLachlan MS. 2004a. Bioaccumulation potential of persistent organic chemicals in humans. *Environ Sci Technol* 38(8):2406-2412.

Czub G, McLachlan MS. 2004b. A food chain model to predict the levels of lipophilic organic contaminants in humans. *Environ Toxicol Chem* 23(10):2356-2366.

Czub G, Wania F, McLachlan MS. 2008. Combining long-range transport and bioaccumulation considerations to identify potential Arctic contaminants. *Environ Sci Technol* 42(10):3704-3709.

Davis JW, Gonsior SJ, Markham DA, Friederich U, Hunziker RW, Ariano JM. 2006. Biodegradation and product identification of [^{14}C]Hexabromocyclododecane in wastewater sludge and freshwater aquatic sediment. *Environ Sci Technol* 40(17):5395-5401.

De Laender F, Van Oevelen D, Frantzen S, Middelburg JJ, Soetaert K. 2010. Seasonal PCB bioaccumulation in an Arctic marine ecosystem: A model analysis incorporating lipid dynamics, food-web productivity and migration. *Environ Sci Technol* 44(1):356-361.

Den Hond E, Govarts E, Bruckers L, Schoeters G. 2009. Determinants of polychlorinated aromatic hydrocarbons in serum in three age classes – Methodological implications for human biomonitoring. *Environ Res* 109(4):495-502.

de Wolf W, Comber M, Douben P, Gimeno S, Holt M, Léonard M, Lillicrap A, Sijm D, van Egmond R, Weisbrod A, Whale G. 2007. Animal use replacement, reduction, and refinement: Development of an integrated testing strategy for bioconcentration of chemicals in fish. *Integr Environ Assess Manag* 3(1):3-17.

Dimitrov SD, Mekenyan OG, Walker JD. 2002. Non-linear modeling of bioconcentration using partition coefficients for narcotic chemicals. *SAR QSAR Environ Res* 13(1):177-184.

EC. 2003. Second edition of the Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances (1996). Parts I through IV. ISBN 92-827-8012-0. Office for Official Publications of the European Communities, Luxembourg.

EC. 2004. European Union system for the evaluation of substances 2.0 (EUSES 2.0). Prepared for the European Chemicals Bureau by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands (RIVM Report no. 601900005). Available via the European Chemicals Bureau, http://ecb.jrc.it.

EC. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18th December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Communities, L369/1-849, 31.12.2006 and amendments, Office for Official Publications of the European Communities, Luxembourg.

EC. 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union L 309/1, 24.11.2009.

EC. 2011. Commission regulation (EU) No 253/2011 of 15 March 2011 amending regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XIII.

ECB. 2000. European Union Risk Assessment Report, Alkanes, C_{10-13}, Chloro-. European Chemicals Bureau, Ispra, Italy.

ECB. 2005. European Union Summary Risk Assessment Report, 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene). European Chemicals Bureau, Ispra, Italy.

ECB. 2007. European Union Risk Assessment Report (Addendum), Alkanes, C_{10-13}, Chloro-. European Chemicals Bureau, Ispra, Italy.

ECB. 2008a. European Union Risk Assessment Report: Anthracene (CAS No: 120-12-7). Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT Assessment. European Chemicals Bureau, Ispra, Italy.

ECB. 2008b. European Union Risk Assessment Report: Coal-tar pitch, high temperature (CAS No: 65996-32-2). Guidance on information requirements and chemical safety assessment. Chapter R.7b: Endpoint specific guidance. European Chemicals Bureau, Ispra, Italy.

ECB. 2008c. European Union Risk Assessment Report: Results of the evaluation of the PBT/vPvB properties of Hexabromocyclododecane (CAS No: 25637-99-4; EINECS No: 247-148-4). PBT Working Group, European Chemicals Bureau, Ispra, Italy. [http://ecb.jrc.ec.europa.eu/documents/existing-chemicals/risk_assessment/report/hbcddreport044.pdf] and fact sheet [http://ecb.jrc.ec.europa.eu/documents/pbt_evaluation/pbt_sum058_cas_25637-99-4.pdf]

ECB. 2008d. Summary fact sheet of anthracene, pure (CAS No: 120-12-7). PBT working group. European Chemicals Bureau, Ispra, Italy.

ECB. 2008e. Results of the evaluation of the PBT/vPvB properties of: Bis(tributyltin) oxide. PBT Working Group. European Chemicals Bureau, Ispra, Italy.

ECB. 2008f. European Union Summary Risk Assessment Report (Addendum), 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene). European Chemicals Bureau, Ispra, Italy.

ECB. 2008g. Summary Fact Sheet PBT working group PBT list No. 56, TC NES subgroup on identification of PBT and vPvB substances results of the evaluation of the PBT/vPvB properties of: Endosulfan.
[http://ecb.jrc.ec.europa.eu/documents/PBT_EVALUATION/PBT_sum056_CAS_115-29-7.pdf]

ECB. 2010. European Union Risk Assessment Report. PBT assessment of musk xylene, 5-tert-butyl-2,4,6-trinitro-m-xylene. Addendum to the final report (2005) of the risk assessment. EUR 24607 EN. European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy.

ECETOC. 2002. Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies. Technical Report No. 85. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2003. Persistence of chemicals in the environment. Technical Report No. 90. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2005a. Risk assessment of PBT chemicals. Technical Report No. 98. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2005b. Guidance for the interpretation of biomonitoring data. Doc. No. 44. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2007a. Workshop on Biodegradation and Persistence. Workshop Report No. 10. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2007b. Intelligent testing strategies in ecotoxicology: Mode of action approach for specifically acting chemicals. Technical Report No. 102. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2009. Collation of existing marine Biodegradation data and its use in environmental risk assessment. Technical Report No. 108. European Centre for Ecotoxicology and Toxicology of Chemicals. Brussels, Belgium.

ECETOC. 2010a. Significance of Bound Residues in Environmental Risk Assessment, 14-15 October 2009, Brussels. Workshop Report No. 17. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2010b. High information content technologies in support of read-across in chemical risk assessment. Technical Report No. 109. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECHA. 2007. Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern. European Chemicals Agency, Helsinki, Finland.

ECHA. 2008a. Guidance on information requirements and chemical safety assessment, chapter R.11: PBT assessment. European Chemicals Agency, Helsinki, Finland.

ECHA. 2008b. Guidance in information requirements and chemical safety assessment, chapter R.7b: Endpoint specific Guidance. European Chemicals Agency, Helsinki, Finland.

ECHA. 2008c. REACH Annex XV dossier proposal for identification of Hexabromocyclododecane as a substance of very high concern. Proposed to identify HBCD as a PBT according to article 57 (d). European Chemicals Agency, Helsinki, Finland. [http://echa.europa.eu/doc/consultations/svhc/svhc_axvrep_sweden_pbt_hbcdd_20083006.pdf] On the basis of this proposal ECHA decided in October 2008 to include HBCD in the list of substances as candidates for Authorisation under REACH. The candidate list is available at: [http://echa.europa.eu/chem_data/authorisation_process/candidate_list_table_en.asp]

EMA. 2006. Guideline on the environmental risk assessment of medicinal products for human use. EMEA/CHMP/SWP/4447/00 corr 1*. European Medicines Agency, London, UK.

Escher B, Fenner K. 2011. Recent advances in environmental risk assessment of transformation products. *Environmental Science and Technology* 45:3835-3847.

EU COMMPS Database. 1998. Proposal for a list of priority substances in the context of the draft Water Framework Directive COM(97)49FIN, 13 August 1998. Report 97/723/3040/DEB/EI prepared for the European Commission DGXI. Fraunhofer Institute, Umweltchemie und Ökotoxicologie, Hannover, Germany.

Euro Chlor. 2002a. Euro Chlor risk assessment for the marine environment. OSPARCOM region – North Sea: Hexachlorobutadiene. Euro Chlor, Brussels, Belgium.

Euro Chlor. 2002b. Euro Chlor risk assessment for the marine environment. OSPARCOM region – North Sea: Hexachlorobenzene. Euro Chlor, Brussels, Belgium.

Euro Chlor. 2011. Euro Chlor risk assessment for the marine environment. OSPARCOM region – North Sea: Alkanes, C_{10-13}, Chloro-. Euro Chlor, Brussels, Belgium.

Evans WC, Fernley HN, Griffiths E. 1965. Oxidative metabolism of phenanthrene and anthracene by soil pseudomonads. The ring-fission mechanism. *Biochem J* 95(3):819-831.

Feijtel T, Kloepper-Sams P, den Haan K, van Egmond R, Comber M, Heusel R, Wierich P, ten Berge W, Gard A, de Wolf W, Niessen H. 1997. Integration of bioaccumulation in an environmental risk assessment. *Chemosphere* 34(11):2337-2350.

Fenner K, Scheringer M, Hungerbühler K. 2003. Joint persistence of transformation products in chemicals assessment: Case studies and uncertainty analysis. *Risk Anal* 23(1):35-53.

Fenner K, Gao J, Kramer S, Ellis L, Wackett L. 2008. Data-driven extraction of relative reasoning rules to limit combinatorial explosion in biodegradation pathway prediction. *Bioinformatics* 24(18):2079-2085.

Forbes VE, Calow P. 2002. Extrapolation in ecological risk assessment: Balancing pragmatism and precaution in chemical controls legislation. *BioScience* 52(3):249-257.

Forbes VE, Calow P, Sibly RM. 2008. The extrapolation problem and how population modeling can help. *Environ Toxicol Chem* 27(10):1987-1994.

Franke C, Studinger G, Berger G, Böhling S, Bruckmann U, Cohors-Fresenborg D, Jöhncke U. 1994. The assessment of bioaccumulation. *Chemosphere* 29(7):1501-1514.

Gatermann R, Hühnerfuss H, Rimkus G, Wolf M, Franke S. 1995. The distribution of nitrobenzene and other nitroaromatic compounds in the North Sea. *Mar Pollut Bull* 30(3):221-227.

Gatermann R, Hühnerfuss H, Rimkus G, Attar A, Kettrup A. 1998. Occurrence of musk xylene and musk ketone metabolites in the aquatic environment. *Chemosphere* 36(11):2535-2547.

Giltrow E, Eccles PD, Winter MJ, McCormack PJ, Rand-Weaver M, Hutchinson TH, Sumpter JP. 2009. Chronic effects assessment and plasma concentrations of the beta-blocker propranolol in fathead minnows (*Pimephales promelas*). *Aquat Toxicol* 95(3):195-202.

Gobas FAPC, Arnot JA. 2010. Food web bioaccumulation model for polychlorinated biphenyls in San Francisco Bay, California, USA. *Environ Toxicol Chem* 29(6):1385-1395.

Gobas FAPC, de Wolf W, Verbruggen E, Plotzke K. 2008. SETAC Pellston Workshop – Revisiting Bioaccumulation Criteria. Society of Environmental Toxicology and Chemistry, 29th Annual North American Meeting, Tampa, Florida, USA, 16-20 November 2008.

Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. *Integr Environ Assess Manag* 5(4):624-637.

Gouin T. 2010. The precautionary principle and environmental persistence: Prioritizing the decision-making process. *Environ Sci Pol* 13(3):175-184.

Gouin T, Mackay D, Webster E, Wania F. 2000. Screening chemicals for persistence in the environment. *Environ Sci Technol* 34(5):881-884.

Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ. 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. *Environ Sci Technol* 42(15):5807-5813.

Hays SM, Aylward LL, LaKind JS. 2008a. Introduction to the biomonitoring equivalents pilot project: Development of guidelines for the derivation and communication of biomonitoring equivalents. *Regul Toxicol Pharmacol* 51(3):S1-S2.

Hays SM, Aylward LL, LaKind JS, Bartels MJ, Barton HA, Boogaard PJ, Brunk C, DiZio S, Dourson M, Goldstein DA, Lipscomb J, Kilpatrick ME, Krewski D, Krishnan K, Nordberg M, Okino M, Tan Y-M, Viau C, Yager JW. 2008b. Guidelines for the derivation of biomonitoring equivalents: Report from the biomonitoring equivalents expert workshop. *Regul Toxicol Pharmacol* 51(3):S4-S15.

Hendriks AJ, van der Linde A, Cornelissen G, Sijm DTHM. 2001. The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight. *Environ Toxicol Chem* 20(7):1399-1420.

Hop H, Pearson T, Hegseth EN, Kovacs KM, Wiencke C, Kwasniewski S, Eiane K, Mehlum F, Gulliksen B, Wlodarska-Kowalczuk M, Lydersen C, Weslawski JM, Cochrane S, Gabrielsen GW, Leakey RJG, Lønne OJ, Zajaczkowski M, Falk-Petersen S, Kendall M, Wängberg S-A, Bischof K, Voronkov AY, Kovaltchouk NA, Wiktor J, Poltermann M, Di Prisco G, Papucci C, Gerland S. 2002. The marine ecosystem of Kongsfjorden, Svalbard. *Polar Res* 21(1):167-208.

Horiguchi F, Nakata K, Ito N, Okawa K. 2006. Risk assessment of TBT in the Japanese short-neck clam (*Ruditapes philippinarum*) of Tokyo Bay using a chemical fate model. *Estuar Coast Shelf Sci* 70(4):589-598.

Houde M, Muir DCG, Kidd KA, Guildford S, Drouillard K, Evans MS, Wang X, Whittle DM, Haffner D, Kling H. 2008. Influence of lake characteristics on the biomagnification of persistent organic pollutants in lake trout food webs. *Environ Toxicol Chem* 27(10):2169-2178.

Howard P, Meylan W, Aronson D, Stiteler W, Tunkel J, Comber M, Parkerton TF. 2005. A new biodegradation prediction model specific to petroleum hydrocarbons. *Environ Toxicol Chem* 24(8):1847-1860.

Hu J, Zhen H, Wan Y, Gao J, An W, An L, Jin F, Jin X. 2006. Trophic magnification of triphenyltin in a marine food web of Bohai Bay, North China: Comparison to tributyltin. *Environ Sci Technol* 40(10):3142-3147.

Huggett DB, Cook JC, Ericson JF, Williams RT. 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Hum Ecol Risk Assess* 9(7):1789-1799.

Huggett DB, Ericson JF, Cook JC, Williams RT. 2004. Plasma concentrations of human pharmaceuticals as predictors of pharmacological responses in fish. In Kümmerer K, ed, *Pharmaceuticals in the environment: Sources, fate, effects and risks*, 2nd ed. Springer-Verlag, Berlin, Germany, pp 373-386.

IEAM. 2009. Special Series: Science-based guidance and framework for the evaluation and identification of PBTs and POPs. *Integr Environ Assess Manag* 5(4):535-711.

IFREMER. 2003. GEMCO final report. Generic estuary modelling system to evaluate transport, fate and impact of contaminants. DEL/EC/301.

IPCS. 1984. Environmental Health Criteria 40: Endosulfan. International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland, 109 p.

IPCS. 2001. Biomarkers in risk assessment: Validity and validation. Environmental Health Criteria 222. International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland.

ISO. 1995. Methods for poorly water soluble chemicals. International Organization for Standardization, Geneva, Switzerland.

Jarvinen AW, Ankley GT. 1999. Linkage of effects to tissue residues: Development of a comprehensive database for aquatic organisms exposed to inorganic and organic chemicals. SETAC Technical Publications Series (99-1). Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA.

Jaworska J, Dimitrov SD, Nikolova N, Mekenyan OG. 2002. Probabilistic assessment of biodegradability based on metabolic pathways: CATABOL system. *SAR QSAR Environ Res* 13(2):307-323.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food web-specific biomagnification of persistent organic pollutants. *Science* 317(5835):236-239.

Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R, Gobas FAPC. 2009. Perfluoroalkyl contaminants in an Arctic marine food web: Trophic magnification and wildlife exposure. *Environ Sci Technol* 43(11):4037-4043.
[http://pubs.acs.org/doi/abs/10.1021/es9003894]

Klasmeier J, Matthies M, MacLeod M, Fenner K, Scheringer M, Stroebe M, Le Gall A-C, McKone TE, van de Meent D, Wania F. 2006. Application of multimedia models for screening assessment of long-range transport potential and overall persistence. *Environ Sci Technol* 40(1):53-60.

Klečka GM, Muir DCG, Dohmen P, Eisenreich SJ, Gobas FAPC, Jones KC, Mackay D, Tarazona JV, van Wijk D. 2009. Introduction to special series: Science-based guidance and framework for the evaluation and identification of PBTs and POPs. *Integr Environ Assess Manag* 5(4):535-538.

Klingmüller D, Watermann B. 2003. TBT – Zinnorganische Verbindungen – Eine wissenschaftliche Bestandsaufnahme. Umweltbundesamt (UBA), Berlin, Germany.

Klöpffer W. 1994. Environmental hazard – Assessment of chemicals and products, Part II: Persistence and degradability of organic chemicals. *Environ Sci Pollut Res* 1(2):108-116.

LaKind JS, Aylward LL, Brunk C, DiZio S, Dourson M, Goldstein DA, Lipscomb J, Kilpatrick ME, Krewski D, Bartels MJ, Barton HA, Boogaard PJ, Lipscomb J, Krishnan K, Nordberg M, Okino M, Tan Y-M, Viau C, Yager JW, Hays SM, 2008. Guidelines for the communication of biomonitoring equivalents: Report from the biomonitoring equivalents expert workshop. *Regul Toxicol Pharmacol* 51(3):S16-S26.

Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F. 2002. Recognition of adverse and nonadverse effects in toxicity studies. *Toxicol Pathol* 30(1):66-74.

McCarty LS. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environ Toxicol Chem* 5(12):1071-1080.

McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. *Environ Sci Technol* 27(9):1719-1728.

Mackay D, Paterson S. 1982. Fugacity revisited. The fugacity approach to environmental transport. *Environ Sci Technol* 16(12):654A-660A.

Mackay D, Wania F. 1995. Transport of contaminants to the Arctic: Partitioning, processes and models. *Sci Total Environ* 160-161:25-38.

Maguire RJ, Tkacz RJ. 1985. Degradation of the tri-n-butyltin species in water and sediment from Toronto Harbor. *J Agric Food Chem* 33(5):947-953.

Masunaga S, Susarla S, Yonezawa Y. 1996. Dechlorination of chlorobenzenes in anaerobic estuarine sediment. *Water Sci Technol* 33(6):173-180.

Meador JP, Adams WJ, Escher BI, McCarty LS, McElroy AE, Sappington KG. 2011. The tissue residue approach for toxicity assessment: Findings and critical reviews from a Society of Environmental Toxicology and Chemistry Pellston Workshop. *Integr Environ Assess Manag* 7(1):2-6.

Meek ME, Sonawane B, Becker RA. 2008. Foreword: Biomonitoring equivalents Special Issue. *Regul Toxicol Pharmacol* 51(3):S3.

Meylan WM, Howard PH. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. *Chemosphere* 26(12):2293-2299.

Miles JRW, Moy P. 1979. Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bull Environ Contam Toxicol* 23(1):13-19.

Moore DRJ, Breton RL, Lloyd K. 1997. The effects of hexachlorobenzene on mink in the Canadian environment: An ecological risk assessment. *Environ Toxicol Chem* 16(5):1042-1050.

Neilson AN. 1999. The handbook of environmental chemistry, volume 3: PAHs and related compounds. Springer-Verlag, Berlin, Germany.

Nfon E, Cousins IT, Broman D. 2008. Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. *Sci Total Environ* 397(1-3):190-204

NRCC. 1975. Endosulfan. National Research Council Canada No. 14098, p 49.

OECD. 1992a. Guidelines for the Testing of Chemicals No. 301. Ready biodegradability. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 1992b. Guidelines for the Testing of Chemicals No. 301. Ready biodegradability, 301 C MITI I test, adopted 17.07.1992. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 1996. Guidelines for the Testing of Chemicals No. 305. Bioconcentration: Flow-through fish test. Organisation for Economic Co-operation and Development, Paris, France. (currently under revision).

OECD. 2002a. Guidelines for the Testing of Chemicals No. 307. Aerobic and Anaerobic Transformation in Soil. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2002b. Guidelines for the Testing of Chemicals No. 308. Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2003. Environment directorate joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology, ENV/JM/MONO(2002)22, OECD Series on Pesticides No. 15, February 2003.

OECD. 2004. Guidelines for the Testing of Chemicals No. 309. Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test. Organisation for Economic Co-operation and Development, Paris, France.

Owen SF, Giltrow E, Huggett DB, Hutchinson TH, Saye JA, Winter MJ, Sumpter JP. 2007. Comparative physiology, pharmacology and toxicology of β-blockers: Mammals versus fish. *Aquat Toxicol* 82(3):145-162.

Owen SF, Huggett DB, Hutchinson TH, Hetheridge MJ, Kinter LB, Ericson JF, Sumpter JP. 2009. Uptake of propranolol, a cardiovascular pharmaceutical, from water into fish plasma and its effects on growth and organ biometry. *Aquat Toxicol* 93(4):217-224.

Patterson M. 2004. Lindane: Analysis of risks to endangered and threatened salmon and steelhead. Environmental Field Branch Office of Pesticide Programs.

Peters A, Crane M, van Wijk D. 2009. Risk assessment for Arctic marine predators. Presentation at the 19th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC). Göteborg, Sweden.

Preuss TG, Hommen U, Alix A, Ashauer R, van den Brink P, Chapman P, Ducrot V, Forbes V, Grimm V, Schäfer D, Streissl F, Thorbek P. 2009. Mechanistic effect models for ecological risk assessment of chemicals (MEMoRisk)—A new SETAC-Europe Advisory Group. *Environ Sci Pollut Res* 16(3):250-252.

Qiu XH, Zhu T, Yao B, Hu JX, Hu SW. 2005. Contribution of dicofol to the current DDT pollution in China. *Environ Sci Technol* 39(12):4385-4390.

Qiu Y-W, Zhang G, Guo L-L, Cheng H-R, Wang W-X, Li X-D, Wai OWH. 2009. Current status and historical trends of organochlorine pesticides in the ecosystem of Deep Bay, South China. *Estuar Coast Shelf Sci* 85(2):265-272.

Ramsay JA, Li H, Brown RS, Ramsay BA. 2003. Naphthalene and anthracene mineralization linked to oxygen, nitrate, Fe(III) and sulphate reduction in a mixed microbial population. *Biodegradation* 14(5):321-329.

RIVM. 1995. Integrated environmental quality objectives for polycyclic aromatic hydrocarbons. RIVM Report No. 679101018.

RIVM. 1996. Initial risk assessment of musk ketone and musk xylene in the Netherlands in accordance with the EU-TGD. RIVM Report No. 601503002.

Royal Haskoning. 2005. Addendum to the risk profile of dicofol, Haskoning Nederland BV Environment.

Ruiz JM, Bachelet G, Caumette P, Donard OFX. 1996. Three decades of tributyltin in the coastal environment with emphasis on Arcachon Bay, France. *Environ Pollut* 93(2):195-203.

Sappington KG, Bridges TS, Bradbury SP, Erickson RJ, Hendriks AJ, Lanno RP, Meador JP, Mount DR, Salazar MH, Spry DJ. 2011. Application of the Tissue Residue Approach in ecological risk assessment. *Integr Environ Assess Manag* 7(1):116-140.

SCC. 2009. Environmentally Classified Pharmaceuticals – January 2009 Edition. Stockholm County Council, Stockholm, Sweden.

Scheringer M. 1996. Persistence and spatial range as endpoints of an exposure-based assessment of organic chemicals. *Environ Sci Technol* 30(5):1652-1659.

Scheringer M, MacLeod M, Wegmann F. 2006. Analysis of four current POP candidates with the OECD P_{ov} and LRTP screening tool. Zürich (CH): ETH Zurich. Internal Report, [www.sust-chem.ethz.ch/downloads]. Accessed 1 June 2009.

Scheringer M, Jones KC, Matthies M, Simonich S, van de Meent D. 2009. Multimedia partitioning, overall persistence, and long-range transport potential in the context of POPs and PBT Chemical assessments. *Integr Environ Assess Manag* 5(4):557-576.

Schwarzbach SE. 1991. The role of dicofol metabolites in the eggshell thinning response of Ring Neck Doves. *Arch Environ Contam Toxicol* 20(2):200-205.

SETAC. 2008. SETAC / Pellston WS: Klečka GM, Muir DCG. Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs: Summary of the Pellston Workshop on 28 January – 1 February 2008, Pensacola, Florida USA. Society of Environmental Toxicology and Chemistry (www.setac.org)

Sijm DTHM, Hermens JLM. 2000. Internal effect concentration: Link between bioaccumulation and ecotoxicity for organic chemicals. In Beek B, ed, *The handbook of environmental chemistry* - Vol 2 Part J – Bioaccumulation: New aspects and developments. Springer-Verlag, Berlin, Germany, pp 167-199.

Smítková H, Huuijbregts MAJ, Hendriks AJ. 2005. Comparison of three fish bioaccumulation models for ecological and human risk assessment and validation with field data. *SAR QSAR Environ Res* 16(5):483-493.

STEP. 2004. Simulation testing of environmental persistence: report of a two-day workshop held in Rotterdam on 4-5th October 2004. Strategies for selecting biodegradation simulation tests and their interpretation in persistence evaluation and risk assessment. Bowmer T, Leopold A, Schaefer E. Hansveit R.

Swackhamer DL, Needham LL, Powell DE, Muir DCG. 2009. Use of measurement data in evaluating exposure of humans and wildlife to POPs/PBTs. *Integr Environ Assess Manag* 5(4):638-661.

Takeuchi I, Miyoshi N, Mizukawa K, Takada H, Ikemoto T, Omori K, Tsuchiya K. 2009. Biomagnification profiles of polycyclic aromatic hydrocarbons, alkylphenols, and polychlorinated biphenyls in Tokyo Bay elucidated by $\delta^{13}C$ and $\delta^{15}N$ isotope ratios as guides to trophic web structure. *Mar Pollut Bull* 58(5):663-671.

TemaNord. 2008. Hexabromocyclododecane as a possible global POP. Nordic Council of Ministers, Copenhagen, DK.

Thompson RS, Stewart KM. 2003. Critical body burdens: A review of the literature and identification of experimental data requirements. Report to the CEFIC LRi BL7549/B. Brixham Environmental Laboratory, AstraZeneca, Brixham, Devon, UK.

Thouand G, Friant P, Bois F, Cartier A, Maul A, Block JC. 1995. Bacterial inoculum density and probability of para-nitrophenol biodegradability test response. *Ecotoxicol Environ Saf* 30(3):274-282.

ToxCast. 2009. The First ToxCast Data Analysis Summit, held May 14-15, 2009, at the EPA Campus in RTP, NC [http://www.epa.gov/ncct/communications.html].

ToxCast™ program. 2007. [http://www.epa.gov/ncct/toxcast/]. US Environmental Protection Agency, USA.

Tsuda T, Nakanishi H, Aoki S, Takebayashi J. 1986. Bioconcentration of butyltin compounds by round Crucian carp. *Toxicol Environ Chem* 12(1-2):137-143.

Tu CM. 1976. Utilization and degradation of lindane by soil microorganisms. *Arch Microbiol* 108(3):259-263.

UNEP. 2004. Guidance for a global monitoring programme for persistent organic pollutants. United Nations Environment Programme, Geneva, Switzerland, 105 p.

UNEP. 2006. Risk Profile on Lindane. United Nations Environment Programme, Geneva, Switzerland.

UNEP. 2007. Risk Management Evaluation on Lindane. United Nations Environment Programme, Geneva, Switzerland.

UNEP. 2010. Report of the Persistent Organic Pollutants Review Committee on the work of its sixth meeting. Addendum: Risk profile on hexabromocyclododecane. October 15, 2010 Advance copy. United Nations Environment Programme, Geneva, Switzerland.

US EPA. 1985. Health Assessment Document for Chlorinated Benzenes, p 6-4. EPA 600/8-84-015F. Final report. US Environmental Protection Agency, Cincinnati, OH, USA.

US EPA. 1998. Reregistration Eligibility Decision – Dicofol. US Environmental Protection Agency, Washington, DC, USA.

US EPA. 2000. Deposition of air pollutants to the Great Waters – 3rd Report to Congress, chapter II. US Environmental Protection Agency, Washington, DC, USA.

US EPA. 2002a. Reregistration Eligibility Decision – Endosulfan. US Environmental Protection Agency, Washington, DC, USA.

US EPA. 2002b. Reregistration Eligibility Decision – Lindane. US Environmental Protection Agency, Washington, DC, USA.

US EPA. 2008. White paper on methods for assessing ecological risks of pesticides with persistent, bioaccumulative and toxic characteristics. Submitted to the FIFRA Scientific Advisory Panel for review and comment. USA Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Environmental Fate and Effects Division, Washington, DC, USA

US EPA. 2009. EPISuite. US Environmental Protection Agency, Washington, DC, USA.

US EPA. 2010. Water quality benchmarks. US Environmental Protection Agency, Washington, DC, USA. [http://www.epa.gov/bpspill/water-benchmarks.html]

van de Meent D, McKone TE, Parkerton TF, Matthies M, Scheringer M, Wania F, Purdy R, Bennett DH. 2000. Persistence and transport potential of chemicals in a multimedia environment. In: Klečka G, Boethling R, Franklin J, Grady L, Graham D, Howard PH, Kannan K, Larson RJ, Mackay D, Muir D, van de Meent D, editors. Evaluation of persistence and long-range transport potential of organic chemicals in the environment. Pensacola (FL): SETAC Press. p 169-204.

van der Meer JR, de Vos WM, Harayama S, Zehnder AJB. 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. *Microbiol Rev* 56(4):677-694.

van Wijk D, Presow S. 2011. Innovation in the assessment of risks of PBTs and POPs. Euro Chlor Abstract 4506, August 25, 2011.

van Wijk D, Chénier R, Henry T, Hernando MD, Schulte C. 2009. Integrated approach to PBT and POP prioritization and risk assessment. *Integr Environ Assess Manag* 5(4):697-711.

van Wijk D, Presow S, Jones A. 2011. Hexachlorobutadiene marine risk assessment for the North Sea and evaluation of secondary poisoning risks. Presented at SETAC, Milan, May 2011.

Verhaar HJM, van Leeuwen CJ, Hermens JLM. 1992. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* 25(4):471-491.

Verschueren K. 1983. Handbook of Environmental Data on Organic Chemicals, 3rd ed. Van Nostrand Reinhold Co, New York, NY, USA, pp 1457.

Walters DM, Fritz KM, Johnson BR, Lazorchak JM, McCormick FH. 2008. Influence of trophic position and spatial location on polychlorinated biphenyl (PCB) bioaccumulation in a stream food web. *Environ Sci Technol* 42(7):2316-2322.

Wan Y, Jin X, Hu J, Jin F. 2007. Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environ Sci Technol* 41(9):3109-3114.

Wania F. 2006. Potential of degradable organic chemicals for absolute and relative enrichment in the Arctic. *Environ Sci Technol* 40(2):569-577.

Wania F, Dugani CB. 2003. Assessing the long-range transport potential of polybrominated diphenyl ethers: A comparison of four multimedia models. *Environ Toxicol Chem* 22(6):1252-1261.

Webster E, Mackay D, Wania F. 1998. Evaluating environmental persistence. *Environ Toxicol Chem* 17(11):2148-2158.

Wegmann F, MacLeod M, Scheringer M. 2007. POP candidates 2007: Model results on overall persistence and long-range transport potential obtained with the OECD P_{ov} and LRTP screening tool – Working paper. [www.sust-chem.ethz.ch/downloads]. Accessed 20 July 2009.

Whelan MJ, Estrada E, van Egmond R. 2004. A modelling assessment of the atmospheric fate of volatile methyl siloxanes and their reaction products. *Chemosphere* 57(10):1427-1437.

WHO. 1990. Environmental Health Criteria 116, Tributyltin compounds. International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland.

Wu RS, Chan AK, Richardson BJ, Au DW, Fang JK, Lam PK, Giesy JP. 2008. Measuring and Monitoring Persistent Organic Pollutants in the Context of Risk Assessment. *Mar Pollut Bull* 57(6-12):236-244.

Yamamoto J, Yonezawa Y, Nakata K, Horiguchi F. 2009. Ecological risk assessment of TBT in Ise Bay. *J Environ Manage* 90(Suppl 1):S41-S50.

Yang Y, Li D, Mu D. 2008. Levels, seasonal variations and sources of organochlorine pesticides in ambient air of Guangzhou, China. *Atmos Environ* 42(4):677-687.

Zoeteman BCJ, Harmsen K, Linders JBHJ, Morra CFH, Slooff W. 1980. Persistent organic pollutants in river water and ground water of the Netherlands. *Chemosphere* 9(4):231-249.

APPENDIX A: Overview of regulatory criteria for PBT, vPvB and POP substances (adapted from van Wijk *et al*, 2009)

Regulatory Framework	Persistence	Bioaccumulation	Toxicity	Long-range transport potential
EU PBT Criteria[1]	Half-life in marine water > 60 days or Half-life in fresh or estuarine water > 40 days or Half-life in marine sediment > 180 days or Half-life in freshwater sediment > 120 days or Half-life in soil > 120 days	BCF aquatic > 2000	Long term NOEC or EC_{10} for marine or freshwater organisms < 0.01 mg/L or Meets criteria for carcinogen cat. 1A or 1B[2] or Mutagen cat. 1A or 1B[2] or Toxic to reproduction cat. 1A, 1B or 2[2]	
EU vPvB Criteria[1]	Half-life in marine or freshwater > 60 days or Half-life in marine, freshwater or estuarine sediment > 180 days or Half-life in soil > 120 days	BCF > 5000		
OSPAR PBT Criteria[3]	Not readily biodegradable or Half-life in water > 50 days	log K_{ow} ≥ 4 or BCF ≥ 500	Acute aquatic toxicity $L(E)C_{50}$ ≤ 1 mg/L and long-term NOEC ≤ 0.1 mg/L or mammalian toxicity: CMR or chronic toxicity	
US EPA Toxics Release Inventory Reporting[4]	Half-life ≥ 2 months in soil, sediment, or water or Half-life ≥ 2 days in air	BAF or BCF ≥ 1,000		
US EPA New Chemicals Program[5]			Develop toxicity data where necessary based upon various factors, including concerns for persistence, bioaccumulation, other physico-chemical factors, and toxicity based on existing data	
- control action pending testing	Transformation half-life > 2 months	BCF > 1000		
	Transformation half-life > 6 months	BCF ≥ 5000		
- ban pending testing				

[1] Commission Regulation (EU) No 253/2011 of 15 March 2011 amending Regulation (EC) No 1907/2006.

[2] According to Regulation EC No. 1272/2008 (CLP).

[3] Ospar Convention for the Protection of the Marine Environment of the North-East Atlantic, Meeting of the Ospar Commission (Ospar) Malahide (Ireland): 27 June – 1 July 2005 Annex 7.

[4] www.epa.gov/fedrgstr/EPA-WASTE/1999/October/Day-29/f28169.htm

[5] www.epa.gov/fedrgstr/EPA-TOX/1999/November/Day-04/t28888.htm

Regulatory Framework	Persistence	Bioaccumulation	Toxicity	Long-range transport potential
Canada[6]	Half-life in air ≥ 2 days or Half-life in water ≥ 6 months or Half-life in sediment ≥ 12 months or Half-life in soil ≥ 6 months	BAF ≥ 5000 or BCF ≥ 5000 or log K_{ow} ≥ 5	Not set in policy or regulations, set as 1 mg/L (acute aquatic) or 0.1 mg/L (chronic aquatic) as part of categorisation of substances on Domestic Substances List	Subject to transport to remote areas alternative to half-life criteria
US EPA PBT Profiler[7]	Half-life moderate concern: water > 60 days, sediment > 60 days, soil > 60 days Half-life high concern: water > 180 days, sediment > 180 days, soil > 180 days	Moderate BCF > 1000 High BCF > 5000	Moderate chronic aquatic toxicity: NOEC: 0.1-10 mg/L High chronic aquatic toxicity: NOEC < 0.1 mg/L	
UN-ECE POP Protocol[8]	Half-life in water > 2 months or in sediment > 6 months or in soil > 6 months	BCF or BAF > 5000 or log K_{ow} > 5	Potential to adversely affect human health or environment	Vapour pressure < 1000 Pa and half-life in air > 2 days or monitoring data in remote area
UNEP POP Convention[9]	Half-life in water > 2 months or in sediment > 6 months or in soil > 6 months	BCF or BAF > 5000 or log K_{ow} > 5 or monitoring data in biota	Evidence of adverse effect on human health or the environment or toxicity characteristics indicating potential damage to human health or environment	Measured levels far from source or monitoring data in remote area or multimedia modelling evidence and half-life in air > 2 days

[6] As provided in the Toxic Substances Management Policy (1995) and in the Canadian Environmental Protection Act (1999), Persistence and Bioaccumulation Regulations (2000). Criteria used to categorise substances on the Domestic Substances List for priority-setting and to identify substances to be targeted for virtual elimination.

[7] http://www.pbtprofiler.net

[8] http://www.unece.org/env/popsxg

[9] Stockholm Convention on Persistent Organic Pollutants (POPs), as amended in 2009. (http://chm.pops.int/Convention/tabid/54/language/en-GB/Default.aspx).

APPENDIX B: METABOLITES / DEGRADATION PRODUCTS

A brief review of the major, or likely metabolites or transformation products for each of the substances reviewed in Chapter 3 is given below.

Alkanes, C_{10-13}, Chloro-

According to the ECB Risk Assessment Report for alkanes, C_{10-13}, chloro-, degradation pathways for chlorinated paraffins was through stepwise dechlorination (ECB, 2000).

The biodegradation pathway of a fully chlorine substituted, linear, ten-carbon compound was predicted by the task force using the University of Minnesota Pathway Prediction System. This predicted the first two steps the environmental transformation of the substance to be the substitution of the chlorine atoms on the terminal carbons with hydroxyl groups. This was predicted to be a slow process (although a duration was not given). Using the EPISuite programs to predict the biodegradation, bioaccumulation and ecotoxicity of this substance resulted in properties that were either very close to, or met the PBT criteria (primary degradation timeframe = 2.65 – weeks to months, log BCF = 3.29, 96-hr EC_{50} (*algae*) = 0.178 mg/L). The ultimate biodegradation of this substance also seemed relevant, since stepwise dechlorinations would still qualify this species under the umbrella category of alkanes, C_{10-14}, chloro-. The ultimate biodegradability score predicted by BioWin was 1.14, which corresponds to a recalcitrant substance.

As there are potentially a number of SCCPs with a wide range of chlorination, undertaking biodegradation studies on well characterised commercial materials at either end of the chlorination range might help to facilitate interpolation of biodegradability of other ‘intermediate’ products to a certain degree. However, it is not certain what is currently representative of actual substances and whether the positioning of chlorine groups could result in different biodegradation potential.

So if any degradation products are also identified as PBT/vPvBs, then emission characterisation and risk characterisation will have to be carried out on them as for the parent substance.

Anthracene

The biochemical pathway for the aerobic biodegradation of a number of polycyclic aromatic hydrocarbons (PAHs) including anthracene has been extensively investigated. It is understood that the initial step in the aerobic catabolism of a PAH molecule by bacteria uses a

multicomponent enzyme system to oxidise the PAH to a dihydrodiol. These dihydroxylated intermediates may then be processed through either an ortho-cleavage type of pathway, in which ring fission occurs between the two hydroxylated carbon atoms, or a meta-cleavage type of pathway, which involves cleavage of the bond adjacent to the hydroxyl groups, leading to central intermediates such as protocatechates and catechols. These compounds are further converted to tricarboxylic acid cycle intermediates (van der Meer *et al*, 1992).

For anthracene, the intermediate metabolites predicted by the University of Minnesota Pathway Prediction System are 9, 10-anthraquinone and cis-1,2-dihydroanthracene-1,2-diol / cis-9,10-dihydroanthracene-9,10-diol. These predictions are supported by experimental data on bacterial and fungal degradation of anthracene (Evans *et al*, 1965; Bezalel *et al*, 1996). Besides, complete mineralisation of anthracene by soil microorganism consortia has been experimentally determined (Ramsay *et al*, 2003). The BioWin QSAR predicts primary degradation of anthracene and its metabolites in weeks (values above 3), with a slightly faster degradation of the metabolites compared to anthracene. Ultimate degradation is predicted to take place in months. Due to consistent results of predictive modelling approaches and experimental data it can be concluded that anthracene and its metabolites have comparable half-lives in the environment. This approach is consistent with ECHA Guidance (Chapter RII: PBT Assessment) that suggests an approach based on consensus modelling. All available empirical data for the substance of interest (i.e. metabolite) are gathered, the four BIOWIN models (1, 3, 4 and 5) and CATABOL model are run, the BIOWIN half lives are averaged and compared for consistency against the CATABOL results. Finally the empirical and modelled data are then combined using expert judgment to suggest a range of half lives which may be applicable to that substance. For specific classes of chemicals it may also be possible to run specific QSARs, e.g. HCBIOWIN based on hydrocarbons (Howard *et al*, 2005).

Bis(tributyltin) Oxide

Bis(tributyltin) oxide (TBTO) dissociates in aqueous media forming a hydrated tributyltin (TBT) cation. This is the most likely medium of emissions given how the substance is used. The speciation of the tributyltin cation is dependent on the pH, anion content and temperature (WHO, 1990) and tributyltins are present in seawater and normal conditions as three species (hydroxide, chloride and carbonate). According to the Annex XV dossier, tributyltin also fulfils the P, B and T criteria.

Biodegradation of TBT results in hydrated dibutyltin, monobutyltin and inorganic tin (ECB, 2008e; Maguire and Tkacz, 1985). BCF values for dibutyltin dichloride in round Crucian carp (*Carassius carassius grandoculis*) muscle, vertebrae, liver and kidney were 12, 46, 135 and 61, respectively (Tsuda *et al*, 1986). According to a classification scheme (Franke *et al*, 1994),

this suggests that the potential for bioconcentration in aquatic organisms is low. More importantly, these data do not meet the B criteria and so dibutyltin (DBT) compounds cannot be regarded as PBTs.

Bis(tributyltin) oxide may react with sulphide in sediments to produce bis(tributyltin) sulphide (Blunden and Chapman, 1986). Calculations in EPISuite predicted that this substance was not P (primary biodegradation timeframe calculation result: 4.19, i.e. days), but was very toxic and bioaccumulative (log BCF = 4.76, 96-hour - LC_{50} = 0.005 mg/L, fish).

Based on these results the likely breakdown products of bis(tributyltin) oxide are unlikely to possess properties that meet the current PBT criteria.

Decamethylcyclopentasiloxane (D5)

Degradation of D5 and the subsequent degradation products is reviewed within Brooke *et al*, 2009, however, the most significant routes of degradation are provided below:

Degradation of D5 occurs in the atmosphere through reaction with hydroxyl radicals to form hydroxyl substituted silanols which are increasingly water soluble and less volatile as substitution proceeds. As a result, degradation products tend to be washed out of the atmosphere through wet deposition (Whelan *et al*, 2004).

A detailed hydrolysis study was conducted by Dow Corning and summarised in Brooke *et al*, 2009. This study identified the intermediate products of the hydrolysis reaction to be dimethylsiloxane-α, ω-diol oligomers [$HO(Me_2SiO)_nH$] (n = 2–5) with the final hydrolysis product being monomeric dimethylsilandiol [$Me_2Si(OH)_2$]. This final hydrolysis product is then lost through volatilisation and degradation.

Dicofol

Dicofol is a recalcitrant chlorinated pesticide similar in structure to DDT. The metabolism of dicofol is predicted to start with the dihydroxylation of one of its aromatic rings. No information on degradation half-lives is available on the degradation products of dicofol, but metabolites of dicofol will be similar in structure to DDE, the most common DDT metabolite. DDE is recalcitrant (BioWin prediction for ultimate degradation = 1.76) and will persist in the environment. Studies using doves show that some dicofol metabolites (1,1-bis(4-chlorophenyl)-2,2 dichloroethylene) have less pronounced eggshell thinning effects on birds than dicofol itself, showing a decrease in bioaccumulation and subsequent toxicity as a

result of metabolism (Schwarzbach, 1991). Therefore whilst DDE, a known metabolite of DDT, is persistent, the metabolites of dicofol have shown to be less bioaccumulative and toxic than the parent compound, and consequently dicofol, although structurally similar to DDT cannot be assessed using the analogy to DDT.

According to the regulation (EC) No 1107/2009 of the European parliament and of the council of 21st October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC and the article 3.7.2 any active substance, safener or synergist shall only be approved if it is not considered to be a persistent, bioaccumulative and toxic (PBT) substance. The assessment of dicofol was largely based on screening information and led finally to a phase out decision. Further consideration of its degradation kinetics and the fate of the major metabolites may have led to a more informed risk assessment and perhaps a more targeted and limited risk reduction decision.

Endosulfan

Endosulfan mainly transforms to endosulfan sulphate through biotic degradation processes. This substance is reported to be even more persistent than the parent molecule (US EPA, 2002a; IPCS, 1984). The biotic and abiotic degradation half-lives of endosulfan sulphate in water are 11 weeks and 178 days, respectively (Miles and Moy, 1979; Callahan *et al*, 1979). A BCF of 130 has been reported, which compares favourably to the log BCF of 2.08 calculated using BCF/BAF in EPISuite (version 4) (US EPA, 2009). The 48-hour LC_{50} is 1.6 ug/L (guppy) (NRCC, 1975).

According to the data presented, both the P and T criteria are definitely fulfilled for endosulfan sulphate. The bioaccumulation seems much lower and would not be considered to be of concern. However, the very high acute toxicity (1.6 ug/L) should be given detailed attention, as acute effects may very easily occur, in spite of endosulfan sulphate's low potential to bioaccumulate. No more studies are required, since this metabolite is not a PBT but is very toxic. The outcome of the risk assessment will decide whether it should be used. Monitoring for endosulfan sulphate and or model predictions of its environmental concentrations under field conditions would be needed to adequately address the risk of this metabolite.

Hexabromocyclododecane (HBCD)

Experimental data are available showing the primary degradation of HBCD in sediments and anaerobic wastewater treatment plant sludge, probably due to sequential debromination via dihaloelimination where at each step there is the loss of two bromines from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms

(Davis *et al*, 2006). The three main sequential metabolites measured were identified as tetrabromocyclododecene, dibromocyclododecadiene, and cyclododecatriene. The study demonstrated the complete debromination of HBCD, which happened faster under anaerobic conditions. The TC NES subgroup on identification of PBT and vPvB substances concluded in 2008 that cyclododecatriene did not fulfil the P or vP criteria, showing that the final degradation product of HBCD is not a PBT. A possible refinement would include a modelling of the relative velocities of the degradation steps to come to a more accurate estimate of the degradation kinetics.

Hexachlorobenzene

The major transformation product of the anaerobic biodegradation of hexachlorobenzene is pentachlorobenzene, via reductive dechlorination (Masunaga *et al*, 1996). Further degradation occurs through the subsequent removal of chlorides. Pentachlorobenzene has biotic and abiotic degradation half-lives of 194-345 days (soil) and 277 days (air), respectively (Beck and Hansen, 1974; Meylan and Howard, 1993). No degradation was found to occur by hydrolysis (Choudhry *et al*, 1983). The bioaccumulation factor was found to be 260,000 in guppy, based on lipid content (Verschueren, 1983). The lowest acute toxicity value found was a 96-hour LC_{50} value of 0.25 mg/L in the bluegill sunfish (US EPA, 1985). However, QSAR predictions using ECOSAR resulted in a 96-hour LC_{50} of 0.029 mg/L to the saltwater mysid shrimp. Caution should be taken when interpreting the measured toxicity data, as the less chlorinated 1,2,4-trichlorobenzene is recognised by the European Chemicals Bureau as a PBT. This will likely be one of the degradation products of pentachlorobenzene from further transformation steps. A screening assessment could be performed to evaluate the risk of HCB metabolites. However, as for the parent compound, there are large uncertainties associated to metabolite environmental emissions estimation.

Although measured data do not confirm that pentachlorobenzene meets the T criterion, it does however meet the vPvB criteria. 1,2,4-Trichlorobenzene, which is also a likely metabolite of hexachlorobenzene, is also confirmed as a PBT. So, where there are relevant degradation products which are also identified as PBT/vPvBs, then emission characterisation and risk characterisation will have to be carried out on them as for the parent substance.

1,3-Hexachlorobutadiene (HCBD)

HCBD was found to form 1,2,3,4-tetrachlorobutadiene through abiotic degradation (Zoeteman *et al*, 1980). This is the result of a single dechlorination at both terminal carbons of the molecule. The PBT properties of this substance were predicted using EPISuite. The primary

biodegradation prediction resulted in a score of 3.17, corresponding to a timeframe of weeks. The calculated log BCF was 2.13 and the lowest predicted toxicity value was for the marine mysid shrimp (96-hour LC_{50} = 0.161 mg/L).

On the basis of these screening-level predictions using EPISuite QSARs, this major transformation product of HCBD is unlikely to meet the PBT criteria. However, it is recommended to confirm this prediction by experimental testing of this transformation product based on stepwise P, B and T testing, starting with persistence studies. If experimental testing confirms 1,2,3,4-tetrachlorobenzene is a PBT, then emission characterisation will have to be carried out on this as for HCBD. Experiments could be designed to confirm the rate of dechlorination in the environment.

Lindane

Known degradation products of lindane are gamma-2,3,4,5,6-pentachloro-1-hexane, and alpha-, beta- and gamma-3,4,5,6-tetrachloro-1-hexane (Tu, 1976). No experimental data could be located pertaining to the PBT properties of these substances, so the related fate and ecotoxicity endpoints were predicted using EPISuite.

The primary degradation timeframe score of gamma-2,3,4,5,6-pentachloro-1-hexane was 2.98, which corresponds to a period of weeks. A log BCF of 2.03 and a 48-hour EC_{50} (*daphnid*) of 0.716 mg/L were predicted.

The primary degradation timeframe score of the default isomer of 3,4,5,6-tetrachloro-1-hexane was 3.13. This also corresponds to a period of weeks, but slightly less, which can be expected from the reduced number of chlorine atoms attached. A log BCF of 1.91 and a 96-hour LC_{50} (fish) of 1.764 mg/L were predicted.

The degradation products of lindane are not expected to meet the PBT criteria, according to predictions using EPISuite programs, however they may be of significant persistence.

Musk xylene

The abiotic degradation of musk xylene occurs via reduction of the nitro groups. The risk assessment of the metabolites has been discussed previously (section 3.1.3.11).

MEMBERS OF THE TASK FORCE

S. Jacobi (Chairman)	Albemarle B - Louvain-la-Neuve
C. Büche*	Ciba Expert Services (now BASF) CH - Basel
C.V. Eadsforth	Shell UK – Chester
P. Lemaire	Total Fluides F - Paris
M. Leon Paumen	ExxonMobil B - Machelen
I. Malcomber	Unilever UK - Sharnbrook
F. Mastrocco	Pfizer USA - New York
S. Navis	Albemarle B - Louvain-la-Neuve
D. Salvito	RIFM USA - Woodcliff Lake
A. Siebel-Sauer	BASF D - Ludwigshafen
J. Snape	AstraZeneca UK - Brixham
M. Galay Burgos	ECETOC B - Brussels

Aknowledgement: The Task Force is indebted to Chris Hughes (Shell, UK) for his substantial contribution to the report.

* *Resigned from the Task Force when leaving the company end 2009.*

MEMBERS OF THE SCIENTIFIC COMMITTEE

(Peer Review Committee)

F. Lewis (Chairman) Global Platform Lead	Syngenta UK - Jealott's Hill, Bracknell
B. van Ravenzwaay (Vice Chairman) Senior Vice President - Experimental Toxicology	BASF D - Ludwigshafen
R. Bars Team Leader, Toxicology Research	Bayer CropScience F - Sophia Antipolis
D. Farrar Occupational Health Business Manager	Ineos Chlor UK - Runcorn
A. Flückiger Head of Corporate Health Protection	F. Hoffmann - La Roche CH - Basel
H. Greim Institute of Toxicology and Environmental Hygiene	Technical University München D - München
G. Malinverno Global Governmental & Regulatory Affairs	Solvay B - Brussels / I - Milano
L. Maltby Professor, Head of Department	University of Sheffield UK - Sheffield
S. Marshall [a] Environmental Science Leader	Unilever SEAC UK - Bedford
M.L. Meisters Manager Health and Environmental Sciences EMEA	DuPont de Nemours B – Mechelen
C. Money Industrial Hygiene Adviser, Europe	ExxonMobil B - Brussels

[a] Responsible for primary peer review.

MEMBERS OF THE SCIENTIFIC COMMITTEE (cont'd)

M. Pemberton Global Product Integrity Manager	Lucite UK - Billingham
C. Rodriguez Principal Toxicologist, Corporate Central Product Safety	Procter and Gamble B - Strombeek-Bever
L. Rushton Principal Research Fellow	Imperial College London UK - London
D. Salvito Vice president, Environmental Sciences	RIFM USA - Woodcliff Lake
J. Snape Principal Scientist	AstraZeneca UK - Brixham
G. Swaen Epidemiologist, Epidemiology, Health Services	Dow NL - Terneuzen
J. Tolls [a] Director Environmental Safety Assessment	Henkel D - Düsseldorf
S. van der Vies Professor of Biochemistry	Vrije Universiteit Amsterdam NL - Amsterdam
C. van Leeuwen Principal Scientist	KWR Watercycle Research Inst. NL - Nieuwegein
H.-J. Wiegand Product Stewardship, Corporate Environment, Safety, Health, Quality	Evonik D - Essen

[a] Responsible for primary peer review.

ECETOC PUBLISHED REPORTS

Monographs

No. Title

No. 1 Good Laboratory Practice (Published October 1979)
No. 2 A Contribution to Strategy for Identification and Control of Occupational Carcinogens (Published September 1980)
No. 3 Risk Assessment of Occupational Chemical Carcinogens (Published May 1985)
No. 4 Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man (Published October 1982)
No. 5 Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology) (Published December 1983)
No. 6 Acute Toxicity Tests, LD_{50} (LC_{50}) Determinations and Alternatives (Published May 1985)
No. 7 Recommendations for the Harmonisation of International Guidelines for Toxicity Studies (Published December 1985)
No. 8 Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary) (Published June 1986)
No. 9 Assessment of Mutagenicity of Industrial and Plant Protection Chemicals (Published June 1987)
No. 10 Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man (Published August 1987)
No. 11 Eye Irritation Testing (Published June 1988)
No. 12 Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity) (Published November 1989)
No. 13 DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment (Published October 1989)
No. 14 Skin Sensitisation Testing (Published March 1990)
No. 15 Skin Irritation (Published July 1990)
No. 16 Early Indicators of Non-Genotoxic Carcinogenesis (Published June 1991)
No. 17 Hepatic Peroxisome Proliferation (Published May 1992)
No. 18 Evaluation of the Neurotoxic Potential of Chemicals (Published September 1992)
No. 19 Respiratory Allergy (Published August 1993)
No. 20 Percutaneous Absorption (Published August 1993)
No. 21 Immunotoxicity: Hazard Identification and Risk Characterisation (Published September 1994)
No. 22 Evaluation of Chemicals for Oculotoxicity (Published November 1994)
No. 23 Receptor Mediated Mechanisms in Chemical Carcinogenesis (Published December 1995)
No. 24 Risk Assessment for Carcinogens (Published July 1996)
No. 25 Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents (Published February 1996)
No. 26 Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances (Published September 1996)
No. 27 Aneuploidy (Published August 1997)
No. 28 Dose-response and threshold-mediated mechanisms in mutagenesis - Mutation Research Special Issue (Published January 2000)
No. 29 Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment (Published September 2000)
No. 30 Genetic Susceptibility to Environmental Toxicants (Published October 2001)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 482, Issues 1-2, Pages 1-115
www.sciencedirect.com/science/journal/00275107
No. 31 Guidance on Evaluation of Reproductive Toxicity Data (Published February 2002)
No. 32 Use of Human Data in Hazard Classification for Irritation and Sensitisation (Published July 2002)

No. 33 Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemical Substances (Published February 2003)
Toxicology Letters, Volume 138, Issues 1-2
www.sciencedirect.com/science/journal/03784274

No. 34 Toxicogenomics in Genetic Toxicology and Hazard Determination (Published August 2005)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 575, Issues 1-2
www.sciencedirect.com/science/journal/00275107

No. 35 Biomarkers and molecular epidemiology (Published August 2006)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 600, Issues 1-2
www.sciencedirect.com/science/journal/00275107

No. 36 Environmental Genotoxins in Children and Adults (Published August 2006)
Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Volume 608, Issue 2
www.sciencedirect.com/science/journal/13835718

No. 37 Biomarkers in Children and Adults (Published July 2007)
Toxicology Letters, Volume 172, Nos. 1-2
www.sciencedirect.com/science/journal/03784274

No. 38 Toxicity of Engineered Nanomaterials (published May 2009)
Toxicology Letters, Volume 186, Issue 3
http://www.sciencedirect.com/science/journal/03784274

Technical Reports

No. Title

No. 1 Assessment of Data on the Effects of Formaldehyde on Humans (Published January 1979) (Updated by TR No. 6)

No. 2 The Mutagenic and Carcinogenic Potential of Formaldehyde (Published May 1981)

No. 3 Assessment of Test Methods for Photodegradation of Chemicals in the Environment (Published August 1981)

No. 4 The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man (Published June 1982) (Updated by TR No. 17)

No. 5 Toxicity of Ethylene Oxide and its Relevance to Man (Published September 1982)

No. 6 Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2 (Published September 1982)

No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere (Published September 1983)

No. 8 Biodegradation Testing: An Assessment of the Present Status (Published November 1983)

No. 9 Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients (Published December 1983)

No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits (Published February 1984)

No. 11 Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5 (Published March 1984)

No. 12 The Phototransformation of Chemicals in Water: Results of a Ring-Test (Published June 1984)

No. 13 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment (Published March 1984)

No. 14 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health (Published March 1984)

No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values (Published June 1984)

No. 16 A Review of Recent Literature on the Toxicology of Benzene (Published December 1984)

No. 17 The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4) (Published April 1985) (Updated by TR No. 64)
No. 18 Harmonisation of Ready Biodegradability Tests (Published April 1985)
No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment (Published May 1985)
No. 20 Biodegradation Tests for Poorly-Soluble Compounds (Published February 1986)
No. 21 Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment (Published February 1986)
No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity (Published January 1987)
No. 23 Evaluation of the Toxicity of Substances to be Assessed for Biodegradability (Published November 1986)
No. 24 The EEC 6th Amendment: Prolonged Fish Toxicity Tests (Published October 1986)
No. 25 Evaluation of Fish Tainting (Published January 1987)
No. 26 The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride (Published January 1987)
No. 27 Nitrate and Drinking Water (Published January 1988)
No. 28 Evaluation of Anaerobic Biodegradation (Published June 1988)
No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns (Published June 1988)
No. 30 Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available) (Published May 1994)
No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment (Published July 1988)
No. 32 Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data (Published May 1988)
No. 33 Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis (Published February 1989)
No. 34 Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man (Published March 1989)
No. 35 Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments (Published January 1990)
No. 36 Biomonitoring of Industrial Effluents (Published April 1990)
No. 37 Tetrachlorethylene: Assessment of Human Carcinogenic Hazard (Published May 1990)
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens (Published July 1990)
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea (Published July 1990)
No. 40 Hazard Assessment of Chemical Contaminants in Soil (Published April 1992)
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products (Published August 1990)
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products (Published February 1991)
No. 43 Emergency Exposure Indices for Industrial Chemicals (Published March 1991)
No. 44 Biodegradation Kinetics (Published September 1991)
No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis (Published March 1992)
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals (Published May 1992)
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification (Published August 1992)
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition) (Published June 1998)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration (Published December 1992)
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models (Published November 1992)
No. 51 Environmental Hazard Assessment of Substances (Published January 1993)
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity (Published August 1992)

No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8) (Published February 1993)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment (Published August 1993)
No. 55 Pulmonary Toxicity of Polyalkylene Glycols (Published December 1997)
No. 56 Aquatic Toxicity Data Evaluation (Published December 1993)
No. 57 Polypropylene Production and Colorectal Cancer (Published February 1994)
No. 58 Assessment of Non-Occupational Exposure to Chemicals (Published May 1994)
No. 59 Testing for Worker Protection (Published April 1994)
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard (Published May 1994)
No. 61 Environmental Exposure Assessment (Published September 1994)
No. 62 Ammonia Emissions to Air in Western Europe (Published July 1994)
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings (Published February 1995)
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man (Published August 1995)
No. 65 Formaldehyde and Human Cancer Risks (Published May 1995)
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank (Published March 1995)
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs (Published October 1995)
No. 68 Assessment Factors in Human Health Risk Assessment (Published August 1995) (Updated by TR No. 86)
No. 69 Toxicology of Man-Made Organic Fibres (Published April 1996)
No. 70 Chronic Neurotoxicity of Solvents (Published February 1996)
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members) (Published August 1996)
No. 72 Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation (Published June 1997)
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology (Published December 1997)
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals (Published June 1998)
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System (Published December 1998)
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment (Published January 1999)
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank (Published August 1999)
No. 78 Skin Sensitisation Testing: Methodological Considerations (Published December 1999)
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data) (Published June 2001)
No. 80 Aquatic Toxicity of Mixtures (Published July 2001)
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy (Published November 2001)
No. 82 Risk Assessment in Marine Environments (Published December 2001)
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union (Published December 2002)
No. 84 Scientific Principles for Soil Hazard Assessment of Substances (Published July 2002)
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies (Published December 2002)
No. 86 Derivation of Assessment Factors for Human Health Risk Assessment (Published February 2003)
No. 87 Contact Sensitisation: Classification According to Potency (Published April 2003)
No. 88 Environmental Risk Assessment of Difficult Substances (Published June 2003)
No. 89 (Q)SARS: Evaluation of the Commercially Available Software for Human Health and Environmental Endpoints with Respect to Chemical Management Applications (Published September 2003)
No. 90 Persistence of Chemicals in the Environment (Published October 2003)
No. 91 Aquatic Hazard Assessment II (Published November 2003)
No. 92 Soil and Sediment Risk Assessment (Published December 2004)

No. 93 Targeted Risk Assessment (Published December 2004)
No. 94 Whole Effluent Assessment (Published December 2004)
No. 95 The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition) Volume I and Volume II Substance Profiles (Published February 2005)
No. 96 Trends in Children's Health and the Role of Chemicals: State of the Science Review (Published June 2005)
No. 97 Alternative Testing Approaches in Environmental Safety Assessment (Published December 2005)
No. 98 Risk Assessment of PBT Chemicals (Published December 2005)
No. 99 Toxicological Modes of Action: Relevance for Human Risk Assessment (Published July 2006)
No. 100 Contribution to the Methodology for the Development of Acute Exposure Threshold Levels in Case of Accidental Chemical Release (Published July 2006)
No. 101 Guidance for Setting Occupational Exposure Limits: Emphasis on Data-Poor Substances (Published October 2006)
No. 102 Intelligent Testing Strategies in Ecotoxicology: Mode of Action Approach for Specifically Acting Chemicals (Published December 2007)
No. 103 Toxicity of Possible Impurities and By-products in Fluorocarbon Products (Published December 2008)
No. 104 Framework for the Integration of Human and Animal Data in Chemical Risk Assessment (Published January 2009)
No. 105 Evaluation of Cardiac Sensitisation Test Methods (Published October 2009)
No. 106 Guidance on Identifying Endocrine Disrupting Effects (Published June 2009)
No. 107 Addendum to ECETOC Targeted Risk assessment report No. 93 (Published December 2009)
No. 108 Collation of Existing Marine Biodegradation Data and its Use in Environmental Risk Assessment (Published December 2009)
No. 109 High Information Content Technologies in Support of Read-across in Chemical Risk Assessment (Published December 2010)
No. 110 Guidance on Assessment Factors to Derive a DNEL (Published November 2010)
No. 111 Development of guidance for assessing the impact of mixtures of chemicals in the aquatic environment (Published October 2011)
No. 112 Refined Approaches for Risk Assessment of PBT/vPvB Chemicals (Published October 2011)
No. 113 Environmental Impact Assessment for Socio-Economic Analysis of Chemicals: Principles and Practice (Published August 2011)

Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine (Published February 1983)
No. 2 1,4-Dioxane (Published February 1983)
No. 3 Methyl Ethyl Ketone (Published February 1983)
No. 4 Methylene Chloride (Published January 1984)
No. 5 Vinylidene Chloride (Published August 1985)
No. 6 Xylenes (Published June 1986)
No. 7 Ethylbenzene (Published August 1986)
No. 8 Methyl Isobutyl Ketone (Published May 1987)
No. 9 Chlorodifluoromethane (Published October 1989)
No. 10 Isophorone (Published September 1989)
No. 11 1,2-Dichloro-1,1-difluoroethane (HFA-132b) (Published May 1990)
No. 12 1-Chloro-1,2,2,2-tetrafluoroethane (HFA-124) (Published May 1990) (Updated by JACC No. 25)

No. 13 1,1-Dichloro-2,2,2-trifluoroethane (HFA-123) (Published May 1990) (Updated by JACC No. 33)
No. 14 1-Chloro-2,2,2-trifluoromethane (HFA-133a) (Published August 1990)
No. 15 1-Fluoro 1,1-dichloroethane (HFA-141) (Published August 1990) (Updated by JACC No. 29)
No. 16 Dichlorofluoromethane (HCFC-21) (Published August 1990)
No. 17 1-Chloro-1,1-difluoroethane (HFA-142b) (Published August 1990)
No. 18 Vinyl Acetate (Published February 1991)
No. 19 Dicyclopentadiene (CAS: 77-73-6) (Published July 1991)
No. 20 Tris-/Bis-/Mono-(2 ethylhexyl) phosphate (Published May 1992)
No. 21 Tris-(2-butoxyethyl)-phosphate (CAS: 78-51-3) (Published March 1992)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1) (Published January 1993)
No. 23 Polycarboxylate Polymers as Used in Detergents (Published November 1993)
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6) (Published May 1994)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0) (Second Edition) (Published July 1994) (Updated by JACC 46)
No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9) (Published September 1994)
No. 27 *n*-Butyl Acrylate (CAS No. 141-32-2) (Published August 1994)
No. 28 Ethyl Acrylate (CAS No. 140-88-5) (Published September 1994)
No. 29 1,1-Dichloro-1-fluoroethane (HCFC-141b) (CAS No. 1717-00-6) (Published December 1994)
No. 30 Methyl Methacrylate (CAS No. 80-62-6) (Published February 1995)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Published February 1995) (Updated by JACC No. 50)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5) (Published May 1995) (Updated by JACC No. 54)
No. 33 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) (CAS No. 306-83-2) (Published February 1996) (Updated by JACC No. 47)
No. 34 Acrylic Acid (CAS No. 79-10-7) (Published September 1995)
No. 35 Methacrylic Acid (CAS No. 79-41-4) (Published May 1996)
No. 36 *n*-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9) (Published December 1996)
No. 37 Methyl Acrylate (CAS No. 96-33-3) (Published September 1998)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3) (Published June 1999)
No. 39 Tetrachloroethylene (CAS No. 127-18-4) (Published December 1999)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions (Published January 2001)
No. 41 *n*-Butanol (CAS No. 71-36-3) (Published March 2004)
No. 42 Tetrafluoroethylene (CAS No. 116-14-3) (Published December 2003)
No. 43 *sec*-Butanol (CAS No. 78-92-2) (Published December 2004)
No. 44 1, 1, 1, 3, 3-Pentafluoropropane (HFC-245fa) (Published June 2004)
No. 45 1, 1-Difluoroethane (HFC-152a) (CAS No. 75-37-6) (Published September 2004)
No. 46 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) CAS No. 2837-89-0 (Third Edition) (Published November 2004)
No. 47 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2 (Third Edition) (Published May 2005)
No. 48 Hexafluoropropylene (HFP) CAS No. 116-15-4 (Published September 2005)
No. 49 Vinylidene Fluoride CAS No. 75-38-7 (Published November 2005)
No. 50 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Second Edition) (Published January 2006)
No. 51 Synthetic Amorphous Silica (CAS No. 7631-86-9) (Published September 2006)
No. 52 Trifluoroethane (HFC-143a) CAS No. 420-46-2 (Published October 2006)
No. 53 Cyanides of Hydrogen, Sodium and Potassium, and Acetone Cyanohydrin (CAS No. 74-90-8, 143-33-9,151-50-8 and 75-86-5) (Published September 2007)
No. 54 Difluoromethane (HFC-32) CAS No. 75-10-5 (Second Edition) (Published June 2008)

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances (Published October 1994)
No. 9	Styrene Criteria Document (Published June 1995)
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1) (Published July 1996)
No. 11	Ecotoxicology of some Inorganic Borates (Published March 1997)
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0) (Published January 1997)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents (Published August 1997)
No. 14	*n*-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document (Published May 1998)
No. 15	Examination of a Proposed Skin Notation Strategy (Published September 1998)
No. 16	GREAT-ER User Manual (Published March 1999)
No. 17	Risk Assessment Report for Existing Substances Methyl *tertiary*-Butyl Ether (Published December 2003)

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development (Published January 1996)
No. 33	Environmental Oestrogens: A Compendium of Test Methods (Published July 1996)
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals (Published February 1996)
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances (Published May 1997)
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals (Published August 1997)
No. 37	EC Classification of Eye Irritancy (Published December 1997)
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification (Published January 1998)
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach (Published January 1999)
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene (Published October 2000)
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1 (Published January 2000)
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction (Published April 2001)
No. 43	Contact Sensitisation: Classification According to Potency. A Commentary (Published July 2003)
No. 44	Guidance for the Interpretation of Biomonitoring Data (Published November 2005)
No. 45	Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study (Published March 2008)
No. 46	Potency Values from the Local Lymph Node Assay: Application to Classification, Labelling and Risk Assessment (Published December2008)

Workshop Reports

No. Title

No. 1 Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels (Published December 2003)

No. 2 Strategy Report on Challenges, Opportunities and Research needs arising from the Definition, Assessment and Management of Ecological Quality Status as required by the EU Water Framework Directive based on the workshop EQS and WFD versus PNEC and REACH - are they doing the job? 27-28 November 2003, Budapest (Published March 2004)

No. 3 Workshop on the Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff (Published November 2004)

No. 4 Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin (Published October 2004)

No. 5 Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Paris (Published December 2004)

No. 6 Workshop on Chemical Pollution, Respiratory Allergy and Asthma. 16-17 June 2005, Leuven (Published December 2005)

No. 7 Workshop on Testing Strategies to Establish the Safety of Nanomaterials. 7-8 November 2005, Barcelona (Published August 2006)

No. 8 Workshop on Societal Aspects of Nanotechnology. 7-8 November 2005, Barcelona (Published October 2006)

No. 9 Workshop on the Refinement of Mutagenicity/Genotoxicity Testing. 23-24 April 2007, Malta (Published September 2007)

No. 10 Workshop on Biodegradation and Persistence. 26-27 June 2007, Holmes Chapel (Published September 2007)

No. 11 Workshop on the Application of 'Omics in Toxicology and Ecotoxicology: Case Studies and Risk Assessment. 6-7 December 2007, Malaga (Published July 2008)

No. 12 Workshop on Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study. 14-15 April 2008, Barza d'Ispra (Published August 2008)

No. 13 Counting the Costs and Benefits of Chemical Controls: Role of Environmental Risk Assessment in Socio-Economic Analysis. 4 June 2008, Brussels (Published September 2008)

No. 14 Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. 24-25 June 2008, Brussels (Published February 2009)

No. 15 The Probabilistic Approaches for Marine Hazard Assessment. 18-19 June 2008, Oslo (Published June 2009)

No. 16 Guidance on interpreting endocrine disrupting effects. 29-30 June 2009, Barcelona (Published October 2009)

No. 17 Significance of Bound Residues in Environmental Risk Assessment. 14-15 October 2009, Brussels (Published December 2009)

No. 18 The Enhancement of the Scientific Process and Transparency of Observational Epidemiology Studies. 24-25 September 2009, London (Published December 2009)

No. 19 'Omics in (Eco)toxicology: Case Studies and Risk Assessment. 22-23 February 2010, Málaga (Published June 2010)

No. 20 Workshop on Guidance on Assessment Factors to Derive a DNEL. 25 March 2010, Barza d'Ispra (Published December 2010)

No. 21 Risk Assessment of Endocrine Disrupting Chemicals. 9-10 May 2011, Florence (Published November 2011)

No. 22 Workshop on Combined Exposure to Chemicals. 11-12 July 2011, Berlin (Published October 2011)

All ECETOC reports can be downloaded from www.ecetoc.org/publications

Responsible Editor:
Dr. Neil Carmichael
ECETOC AISBL
Av. E. Van Nieuwenhuyse 4 (bte. 6)
B-1160 Brussels, Belgium
VAT: BE 0418344469
www.ecetoc.org
D-2011-3001-218

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) was established in 1978 as a scientific, non-profit making, non-commercial association and counts as its members the leading companies with interests in the manufacture and use of chemicals. An independent organisation, ECETOC provides a scientific forum through which the extensive specialist expertise of manufacturers and users can be harnessed to research, evaluate, assess, and publish reviews on the ecotoxicology and toxicology of chemicals, biomaterials and pharmaceuticals.