Substance Name: Pyrene
EC Number: 204-927-3
CAS Number: 129-00-0

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF

PYRENE

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS PBT[1] (ARTICLE 57D) AND vPvB[2] (ARTICLE 57E) PROPERTIES

Adopted on 12 December 2018

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[1] PBT means persistent, bioaccumulative and toxic

[2] vPvB means very persistent and very bioaccumulative

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FOREWORD

Pyrene belongs to the substance group of Polycyclic Aromatic Hydrocarbons (PAHs) of which many are well-known to be hazardous for human health and the environment. Pyrene does not possess a harmonised classification according to the CLP Regulation (EC 1272/2008).

Until now, several Annex XV dossiers for the identification of substances of very high concern (SVHC) were explicitly based on the properties of PAHs as constituents of concern in the identified substances, such as Anthracene, Anthracene Oils, Coal Tar Pitch High Temperature (CTPHT), Benzo[a]pyrene, Benz[a]anthracene and Chrysene.

Pyrene is a constituent of coal-tar pitch, high temperature (CTPHT). In the Support Document of CTPHT, it has been concluded by the Member State Committee (MSC) that pyrene fulfills the PBT and vPvB criteria of Annex XIII to REACH Regulation (ECHA, 2009). However, pyrene and further PAHs whose properties have already been agreed on by the MSC in the CTPHT SVHC identification process have not yet been proposed for formal SVHC identification and inclusion in the Candidate List.

Pyrene was assessed with respect to PBT/vPvB properties based on the MSC Support Document for identification of CTPHT as SVHC (ECHA, 2009). For the purpose of the present SVHC proposal for pyrene, a supplementary literature search was made. The search identified only few studies not included in the EU Risk Assessment Report on CTPHT (European Commission, 2008) and the Annex XV Transitional Dossier on CTPHT (The Netherlands, 2008). Thus, the assessment of the PBT/vPvB properties in the present dossier and the conclusion that pyrene fulfils the criteria in Article 57 (d) and (e) was based mainly on the information in the Support Document on CTPHT (ECHA, 2009) and supplemented with information from newer studies that are presented as further evidence as they do not trigger a need to modify the conclusions taken by authorities earlier on.

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name: Pyrene

EC Number: 204-927-3

CAS number: 129-00-0

- It is identified the substance as persistent, bioaccumulative and toxic (PBT) according to Article 57 (d) of Regulation (EC) No 1907/2006 (REACH).
- It is identified the substance as very persistent and very bioaccumulative (vPvB) according to Article 57 (e) of Regulation (EC) No 1907/2006 (REACH).

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

The assessment of the PBT/vPvB properties in the present dossier and the conclusion that pyrene fulfils the criteria in Article 57 (d) and (e) were based mainly on the information in the MSC Support Document on CTPHT (ECHA, 2009) and supplemented with information from newer studies. This information was considered together in a weight-of-evidence approach.

Persistence
The available experimental information shows that pyrene degrades very slowly in soils exhibiting half-lives between 127 to 320 days, higher than 180 days (vP criteria according REACH Annex XIII). The soil experimental study conducted under field conditions and the predicted half-life support the low degradation in soils.

It is also assumed that pyrene meets the P and vP criterion in sediment, as in the available simulation study with phenanthrene the half life meets the P and vP criterion. Considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in sediment are proportionally related to the octanol-water partition coefficient (Kow), the half-life of pyrene should meet P and vP in sediment as well.

Thus, pyrene fulfills the criteria for P and vP of REACH Annex XIII for soil and sediment.

Bioaccumulation
BCF values higher than 5 000 L/kg (vB criteria according REACH Annex XIII) have been measured in four studies with molluscs (6 430 to 77 000 L/kg), two studies with crustacean (12 300 to 166 000 L/kg) and one study with an oligochaete species (6 688 L/kg).

Thus, pyrene fulfils the B and vB criteria of REACH Annex XIII.

Toxicity
Based on the available information from long-term studies with aquatic organisms, the lowest EC_{10} value was reported for the mollusc *Crassostrea gigas* exposed to UV radiation. The resulting EC_{10} of 0.5 µg/L shows that pyrene fulfils the T criteria (NOEC or EC_{10} for marine or freshwater organisms is less than 0.01 mg/L) of REACH Annex XIII.

Overall conclusion

In conclusion, pyrene meets the criteria for a PBT and vPvB substance according to Article 57 (d) and (e) of REACH Regulation by comparing all relevant and available information according to Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Registration dossiers submitted for the substance: Yes

Justification

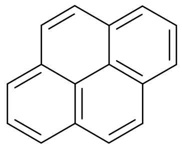
1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	204-927-3
EC name:	Pyrene
CAS number (in the EC inventory):	129-00-0
CAS number: **Deleted CAS numbers:**	-
CAS name:	-
IUPAC name:	Pyrene
Index number in Annex VI of the CLP Regulation	No harmonised classification
Molecular formula:	$C_{16}H_{10}$
Molecular weight range:	202.2506 g.mol^{-1}
Synonyms:	pyren benzo[d,e,f]phenanthrene, beta-pyrene, benzophenantrene

Structural formula:



1.2 Composition of the substance

Name: Pyrene

Description: Pyrene belongs to the group of Polycyclic Aromatic Hydrocarbons (PAHs). Pyrene is generally not produced intentionally but it may occur as a constituent in UVCBs[3] – often together with other PAHs - mainly derived from the coal and petroleum stream. The dossier addresses the substance pyrene as a substance itself.

Substance type: mono-constituent

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[3] Substances of Unknown or Variable composition, Complex reaction products or Biological materials

1.3 Physicochemical properties

Table 2: Overview of physicochemical properties

Property	Description of key information	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa		Solid	Yaws, 2015
Melting/freezing point		145.3°C	ECHA dissemination site
Boiling point		360°C	ECHA, 2009
Vapour pressure		$1.0\ .10^{-3}$ Pa at 25°C	ECHA, 2009
Density		$1.209\ Kg.L^{-1}$ at 20°C	ECHA dissemination site
Water solubility		$0.134\ mg.l^{-1}$at 25°C	ECHA dissemination site
Partition coefficient n-octanol/water (Log value)		5.17 at 25°C	ECHA, 2009
		5.43 at 30°C	ECHA dissemination site
Flash point	According to CSN EN ISO 2592/2002	224.0°C at 101.325 kPa	ECHA dissemination site
Viscosity	According to OECD Test Guideline 114 (Viscosity of Liquids)	$1.8\ mm^2.s^{-1}$ at 150 °C	ECHA dissemination site
Henry's constant		$1.4\ Pa\ m^3/mol$ at 25 °C	ECHA, 2009

2. Harmonised classification and labelling

No harmonised classification for pyrene.

3. Environmental fate properties

3.1 Degradation

The data provided on the degradation of pyrene in the Support Document for identification of CTPHT as SVHC (ECHA, 2009) are not assessed or discussed again in this dossier but included in order to have access to the full dataset for assessment (flagged by *italic print*). Additional information is available in the EU risk assessment report on CTPHT (EC, 2008) and the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008).

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

The stability to hydrolysis of PAHs has been already assessed in the Support Document for identification of CTPHT as SVHC (ECHA, 2009).

Considering that pyrene does not present functional groups that result in hydrolysis, the substance is expected to be *hydrolytically stable in aquatic systems*. The Support Document furthermore states as a result that *hydrolysis does not contribute to the degradation of PAHs under environmental conditions.*

3.1.1.2 Oxidation

The oxidation of PAHs was summarised and discussed in the Support Document for identification of CTPHT as SVHC (ECHA, 2009) as follows:

In the atmosphere, the PAHs are either gas phase or particle-associated. It has been shown that the 2-4 ring PAHs with vapour pressure higher than or equal to 10^{-4} Pa are mostly gas phase-related and PAHs of 4 rings or more with vapour pressure below 10^{-4} Pa are particle-associated. In the gas phase PAHs are oxidised by atmospheric hydroxyl (OH) and nitrate radicals and ozone, whereas the particle-associated PAHs are expected to be degraded by direct photolysis and by reaction with ozone (The Netherlands, 2008).

In the atmosphere, pyrene can be partitioned in the gas phase and also adsorbed to the particle phase. In the gas phase, pyrene can undergo oxidation in the presence of OH radicals exhibiting low lifetimes between a few hours to less than one day (The Netherlands, 2008). Pyrene can be also adsorbed to the particle phase. Indeed, up to 61.4% of atmospheric pyrene has been observed to appear in particle phase (The Netherlands, 2008).

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 Phototransformation in air

The photolysis of PAHs in the atmosphere was already assessed in the EU risk assessment report (EC, 2008) and also discussed in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008). The Support Document for identification of CTPHT as SVHC (ECHA, 2009) summarises the previous assessments as follows:

Photolysis in the troposphere results in the formation of reactive hydroxyl (OH) and nitrate (NO_3) radicals and ozone (O_3), which reacts as oxidizing agent with organic compounds, like PAHs. These radical and ozone reactions comprise mainly degradation of gas-phase PAHs (Calvert et al., 2002). Particle-associated PAHs are expected to degrade in air predominantly via direct photolysis by light with a wavelength < 290 nm (Kamens et al., 1988), although reaction with ozone will also occur (Peters and Seifert, 1980; Grosjean et al., 1983; Pitts et al., 1986; Coutant et al., 1988).

As stated earlier, assuming that pyrene can also be associated to the particle phase in the atmosphere, it is expected that it degrades in air predominantly via direct photolysis, although reaction with ozone will also occur (the Netherlands, 2008). Furthermore, it is also mentioned from the study of Behymer and Hites, 1988, cited in the Netherlands, 2008, that degradation rate of PAHs, is related to the type of particle to which they are adsorbed. The Annex XV transitional dossier (The Netherlands, 2008) showed representative lifetimes of some surface-adsorbed PAHs with respect to photolysis. For pyrene, representative lifetimes between 3.7 hours and 4.8 days are found under representative conditions of a cloudless sky.

3.1.1.3.2 Phototransformation in water

Following the conclusions regarding PAHs in the Support Document for identification of CTPHT as SVHC (ECHA, 2008), an aquatic phototransformation of pyrene in the first few centimeters layer of the water column is expected.

Due to the number of factors that affect photodegradation rates, this process is not generally considered in the persistence assessment for substances registered under REACH. Further discussion on photodegradation is provided in Chapter R.7b of the Guidance on IR&CSA.

According to Castro-Jiménez and de Meent (2011), light absorption in natural water is significantly slower than measured in laboratory water with photo degradation occurring around 30 times more slowly for typical fresh water, 400 times more slowly for typical coastal sea water, and 500 times more slowly for ocean water. These authors also conclude that the "contribution of photodegradation in water to overall degradation is significant only for substances that reside in water to a considerable extent". They highlight that many substances reside in sediment and soil, rather than in water.

Therefore, aquatic photodegradation is not considered to have a significant impact on the overall persistency of pyrene in the environment.

3.1.1.3.3 Phototransformation in soil

Following the conclusion regarding phototransformation of PAHs in the Support Document for identification of CTPHT as SVHC (ECHA, 2008), a significant phototransformation of pyrene in soils is not expected, due to the limited exposition to light. Thus, photodegradation is not considered a relevant degradation process in soils.

3.1.1.4 Summary on abiotic degradation

In the atmosphere, degradation of pyrene by oxidation is expected when it is in the gas phase, as well as by direct photolysis when it is adsorbed to the particle phase. Furthermore, depending on the type of particles that the substance is bound to, the degradation of the substance by photolysis can vary between a few hours to days. In water, the substance is expected to be hydrolytically stable as the substance does not present functional groups that result in hydrolysis. Photodegradation of pyrene in water is not expected to be a relevant removal pathway because it only occurs in the first few centimeters layer of the water column. This degradation process is also not relevant in soils due to the limited exposure to light.

This conclusion was already drawn in the support document for identification of CTPHT as SVHC (ECHA, 2009).

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water and sediments

The biodegradation in water was already assessed in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008) and summarised in the Support Document for identification of CTPHT as SVHC (ECHA, 2009), thus this section will not be assessed again within this dossier.

Experimental information for biodegradation in water has demonstrated that PAH substances with up to four aromatic rings are biodegradable under aerobic conditions, but that biodegradation rates of PAHs with more than four aromatic rings, are very low (The Netherlands, 2008).

In general, the biodegradation rates decrease with increasing number of aromatic rings. This correlation has been attributed to factors like the bacterial uptake rate and the bioavailability. The bacterial uptake rate has been shown to be lower for the higher molecular weight PAHs as compared to the PAHs of lower molecular weight. This may be *due to the size of high molecular weight members, which limits their ability to cross cellular membranes. In addition, bioavailability is lower for higher molecular PAHs due to adsorption to organic matter in water and sediment. It has further been shown that half-lives of PAHs in estuarine sediment are proportionally related to the octanol-water partition coefficient (Kow) (Durant et al. (1995) cited in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008)).*

Mackay et al. (1992) estimated half-lives in the different environmental compartments based on model calculations and literature research. The calculated half-lives of pyrene in water were in the range of 42 to 125 days, and were estimated to be longer than 1250 days for the sediment compartment (ECHA, 2009).

During the public consultation, references of seven studies related to the degradation of pyrene in water were provided (Birch et al., 2018, Concawe, 2012, Martin et al., 2017, Brakstad et al., 2018a, 2018b, Loftus et al., 2018, Ribicic et al., 2018). The studies have been assessed for their reliability and relevance according to OECD and ECHA guidelines (R11 and R7b). They have not been considered suitable for the P assessment as they present methodological limitations, as for example the addition of mineral media in the experiments, the origin of the samples that could be considered as pre-adapted to PAHs, absence of abiotic control and reference substance, the use of dispersant in the experiments, absence of information about the dissolved pyrene in the experiments, use of internal markers for the determination of half- life. In the case of the study of Martin et al.,2017 regarding ready biodegradation test, as pyrene was not tested, the information have not been further considered in the current assessment.

In addition, the results of one poster (Hammershoj et al., 2018) related to the degradation of mixtures was transmitted. As pyrene was not tested, the information have not been further considered in the current assessment.

At present pyrene has not been tested in a sediment simulation study (OECD 308). However, during the public consultation a summary of such a study was provided in which the degradation of phenanthrene was studied (Meisterjahn et al. 2018). When converted to 12°C, the half-lives observed were higher than the vP criterion. Considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in estuarine sediment are proportionally related to the octanol-water partition coefficient (Kow) (Durant et al. (1995) cited in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008)), it can be assumed that pyrene also meets the P and vP criterion in sediment.

3.1.2.2 Biodegradation in soil

The biodegradation in soil was already assessed in the Support Document for identification of CTPHT as SVHC (ECHA, 2009) as follows:

Biodegradation rates of PAHs in soil depend on several factors related to the soil type, including pH, moisture content, nutrients, oxygen, and the diversity of the soil microbial population. Various species (bacteria, fungi, yeasts and algae) are known to degrade PAHs in soil (The Netherlands, 2008). It has been shown that the number of PAH-degrading microorganisms and the degradation capacity is higher in PAH-contaminated soils than in pristine soils, something explained by the development of an adapted soil microbial community. Several studies have also been demonstrated enhanced PAH degradation rates when the soil had been enriched with isolated PAH-degrading microorganisms ([…] The Netherlands, 2008). On the basis of a comparison between two studies (Wild et al. 1991 and Wild and Jones, 1993) it was illustrated that the half-lives observed under laboratory conditions can be much shorter than those obtained from long-term field studies. This was attributed by the authors to the more optimal conditions (temperature, moisture content, nutrient and oxygen supply) applied in the laboratory tests.

Furthermore the Support Document for identification of CTPHT as SVHC (ECHA, 2009) discusses "aging" for PAHs as follows:

"'Aging' is a phenomenon associated with increased residence time of PAHs in soil, which can further decrease the bioavailability of PAHs in the terrestrial environment. Freshly spiked PAHs are more readily desorbed and thus more bioavailable than PAHs that have been in soil or sediment for a longer period of time (The Netherlands, 2008). This means that studies involving artificially added PAHs (e.g. ^{14}C-labelled) often result in biodegradation rates much higher than rates observed for the same substances present in soil as part of a contamination by coal tar."

In the case of pyrene, the study of Wild *et al*. (1991) measured a half-life of 8.5 years in a field experiment with soils enriched with PAH-contaminated sludge, whereas the study of Wild and Jones (1993) derived half-lives for sludge applied pyrene of 127-320 days in their microcosm study with three soil types (sandy loams, forest soil, and roadside soil) conducted at a range of temperature between 20 and 30°C. When pyrene was spiked to soil the half-life was lower, i.e. 51 days. However, as the abiotic loss, due to e.g. volatilization, was high (65%), no reliable half-life can be determined. Nevertheless, when this DT50 is converted to 12°C it will be higher than the P criterion.

Regarding the information provided in the studies by Wild and Jones (1993) and Wild et al. (1991), it should be noted that the MSC has already considered their use in the assessment of CTPHT as SVHC, and hence also for pyrene. In agreement with MSC conclusions made within the CTPHT assessment, the SVHC dossier submitter evaluated the study Wild et al. (1991) as a most reliable evidence of persistency of CTPHT (thus also for pyrene), which is suitable for PBT assessment of pristine environment. Mackay *et al*. (1992) in their calculations predicted half-life values of PAHs for the soil compartment. For pyrene, the calculated half-life was in the range of 420-1250 days.

During the public consultation, a reference to a soil degradation study was transmitted (Sigmund et al, 2018). This study has been assessed for its reliability and relevance according to OECD and ECHA guidelines (R11 and R7b). It is noted that the study Sigmund et al, 2018 is not comparable to the study of Wild and Jones, 1993 due to methodological differences. The experimental objective was to investigate the effects of compost amendment, both with and without biochar on PAHs and NSO-PAH degradation in soils. The soil samples were spiked with 25 mg of pyrene/kg of soil, and additionally spiked with 100 mg/kg of Zn and 10 mg/kg of Cd, heavy metals. This may cause changes in bacterial community and their capacity to biodegrade PAHs. The study was conducted for 120 days with non-radiolabelled pyrene, no information about the recovery of the substance (formation of NER) is available in the publication. Furthermore, the study did not performed an abiotic control. The data presented in the publication for the half-life is limited to a graphical representation making the determination of the real half-life value difficult. Due to methodological limitations, no reliable half-life can be determined from this study.

Harmsen and Rietra, 2018, reference provided during the public consultation, performed a long-term study on soil and sediment focusing on biodegradation of PAHs and total petroleum hydrocarbons (TPH), which has been monitored on seven experimental fields during periods up to 25 years. The study took place on experimental fields at Kreekraksluizen, situated in the Netherlands. Landfarms were initiated on semi-field scale in 1990. About 50 cm of dredged sediment was applied and the layer thickness of the dewatered sediments was about 30 cm. The sediments were intensively treated to stimulate biodegradation during the first years. Treatments used were cultivation, no cultivation, adding wood chips and adding sludge from a sewage system adapted to PAHs. In 1993 the sediment from the different experimental fields were combined into two new experimental fields containing sediments that originated from two harbors in a thicker layer. These sediments were further treated in a passive way (passive landfarming), only allowing vegetation to grow to create aerobic conditions and were followed for 20 years.

The bioavailable fraction was measured using Tenax extraction. The study detailed the remaining concentration of every PAHs measured and also pyrene after 7.2 years and 22 years. The initial concentration of pyrene was 79 mg/kg, after 7.2 years, the remaining amount was estimated being 2.1 mg/kg and after 22 years, estimated of being 0.9 mg/kg. In other sediment from harbour in Wemeldinge were we had an initial concentration of pyrene of 10 mg/kg, after 7.2 years it remains 6.6 mg/kg and after 22 years 1.9 mg/kg. It is observed that the degradation rate of pyrene may depend on the initial concentration and the certain fractions can remain for long period of time in the environment (Harmsen and Rietra, 2018).

3.1.2.3 Summary and discussion on biodegradation

For water, Mackay *et al*. (1992) predicted long half- lives in the range of 42 to 125 days. It is assumed that pyrene meets the P and vP criterion in sediment, as in the available simulation study with phenanthrene the half life meets the P and vP criterion. Considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in sediment are proportionally related to the octanol-water partition coefficient (Kow), the half life of pyrene should meet de P and vP in sediment as well. The half-life predicted by Mackay *et al*. (1992) support the persistency of pyrene in the sediments (half-life> 1250 days).

Regarding the soil compartment, experimental data on microcosm and under field conditions are available from the studies of Wild and Jones (1993) and Wild *et al*. (1991) in which pyrene is applied via sludge. The results show dissipation half-life values for pyrene in the range of 127 to 320 days under microcosm conditions and a half-life of 8.5 years in the field experiment. Furthermore, Mackay *et al*. (1992) predicted half-life for pyrene on the soil compartment in the range of 420-1250 days. A study of Harmsen and Rietra, 2018, suggests that degradation rates of pyrene may depend on the initial concentration and that a certain fraction can remain for a long period of time in the environment.

It is concluded that, based on the available data, pyrene degrades very slowly in sediments and soils.

3.1.3 Summary and discussion of degradation

In the atmosphere, pyrene can be partitioned in the gas phase and also adsorbed in the particle phase. Degradation in the gas phase by oxidation in the presence of OH radicals takes place between a few hours to less than one day. However, pyrene may be more stable in the particulate phase than in the gas phase. Pyrene degradation by photolysis (in the particulate phase) can vary between a few hours to less than 5 days.

In the water and soil compartments, photolysis is only relevant in the upper few centimeters of the water column and the upper few millimeters of the soil. Thus, photodegradation is not considered as a relevant degradation process in water and terrestrial environments.

In general, PAHs are considered as chemically stable substances, with no functional groups that result in hydrolysis in the water and soil compartments. Therefore, pyrene is considered as hydrolytically stable.

Estimated half-lives for pyrene in water ranged between 42 - 125 days and for sediments longer than 1250 days. In view of the fact that phenanthrene meets the P and vP criterion in a sediment simulation study (Meisterjahn et al. 2018), it is assumed that pyrene will meet P and vP criterion as well considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in sediment are

proportionally related to the octanol-water partition coefficient (Kow) (Durant et al. (1995) cited in The Netherlands, 2008).
The available experimental data show that pyrene exhibits a low biodegradation in the soil compartment when pyrene is applied via sewage sludge. The study conducted under microcosm conditions by Wild and Jones (1993) reported half-life values of 127 to 320 days or even higher under field conditions, (Wild *et al*., 1991). A study of Harmsen and Rietra, 2018, suggest that the degradation rate of phenanthrene may depend on the initial concentration and that a certain fraction can remain for a long period of time in the environment.

The available information allow to conclude that pyrene meets the P and vP criteria for sediments and soil.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

In the Support Document for identification of CTPHT as SVHC (ECHA, 2009) the adsorption properties of PAHs are described as follows: *"A linear relationship between Kow and the organic carbon-water partitioning coefficient Koc has been demonstrated for PAHs in sediments and soil. The Log Kow values from 4.6 to 6.6 can be translated as a high potential for partitioning to soils and sediments. Partitioning processes like adsorption to airborne particulate matter, as well as accumulation in sludge during wastewater treatment, have been demonstrated especially for high molecular weight PAHs (The Netherlands, 2008)."*

Based on the Log K_{ow} of 4.98 for pyrene, reported in the Annex XV Transitional Dossier on CTPHT (The Netherlands, 2008), the coefficient of partitioning between organic carbon and water, Log K_{OC}, has been estimated at 4.77 (The Netherlands, 2008) and it is concluded that pyrene has a high potential to adsorb to particles in the environment.

In the registration dossier (ECHA, 2009) a range of 4.1-5.9 is reported for Log K_{OC}, but no details are provided in the methodology.

3.2.2 Volatilisation

Pyrene exhibits a vapour pressure of 1.0 x 10^{-3} Pa at 25°C and a Henry's law constant of 1.4 Pa m^3/mol at 25°C (Mackay *et al*., 2006, cited by The Netherlands, 2008). Thus, some volatilisation is expected from water or soil surfaces.

3.2.3 Distribution modelling

Mackay Level III fugacity modelling was done using EPI Suite (version 4.11) with default values of environmental emission rates (equal and continuous release to water, soil and air). Calculation revealed a distribution of pyrene mainly to the soil compartment followed by the sediment and water phase. The air compartment is not expected to be a relevant route of distribution.

Table 3: Fugacity Model Calculation (EPI Suite, version 4.11) of Pyrene.

Distribution to:	Mass amount (percent)

Air	0
Water	7.64
Soil	61.8
Sediment	30.5

3.2.4 Summary and discussion of environmental distribution

Pyrene exhibits a high potential to adsorb to organic matter and some volatilisation from water surfaces. Furthermore, according to fugacity modelling, pyrene is expected to be mainly distributed in the soil compartment followed by the sediment and water compartments. The air compartment is not expected to be a relevant route of distribution.

3.3 Bioaccumulation

3.3.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

Several studies on bioaccumulation in aquatic organisms have been assessed in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008) and reliable selected studies were summarised in the Support Document for identification of CTPHT as SVHC (ECHA, 2009). Furthermore, the RIVM (Bleeker and Verbruggen, 2009) also evaluated available data on bioaccumulation of PAHs in aquatic organisms, including pyrene, and examined their reliability. The studies reported in both reports were not assessed again within this dossier. In addition, a review of literature was carried out in June 2018 and additional data are presented in this dossier.

The support document for identification of CTPHT as SVHC (ECHA, 2009) summarised the results of studies conducted with fish for pyrene as follows:

Jonsson et al., 2004 exposed the fish Cyprinodon variegatus for 36 days to pyrene in continuous flow system with seawater, followed by 8 days of depuration. The BCF values calculated by kinetic approach were 97 and 147 for the low (7.57 µg/L) and the high concentrations (72.31 µg/L) tested. For this experiment a reliability of 1 was attributed (ECHA,2009).

RIVM (Bleeker and Verbruggen, 2009) calculated by kinetic approach a BCF of 1 474 L/kg (after lipid normalisation of 5%) for *Fathead minnows* from the results of the study of Carlson et *al*., 1979 and attributed to the study a validity of 2. In the study, fish were exposed to a series of PAHs via flow through conditions for 28 days followed by 5 days of depuration.

The study of De Voogt *et al*., 1991 reported BCF values from two experiments conducted with *Poecilia reticulate*. In the first study, fish were exposed to pyrene during 48 hours on static conditions. The authors calculated a BCF of 4 810 ± 2 860 L/kg using the Barnejee method. In the RAR, a reliability of 3 was attributed for this study (EC 2008). Furthermore, the RAR of CTPHT (EC, 2008) determined a mass balance for pyrene from the results of the static experiments and derivated another BCF of 2 700 L/kg and they attributed a reliability of 2 for this study. For comparison the authors conducted a 7 days semi- static study and a BCF value of 11 300 L/kg was calculated. The BCF was determined by dividing the final concentration in fish by the average concentration in water during the last renewal period. For this experiment a reliability of 2 (valid with restrictions) was attributed (EC, 2008).

Nevertheless, the results of the experiments conducted by De Voogt et *al*., 1991 were re-assessed in depth by RIVM (Bleeker and Verbruggen, 2009), and were considered as inconclusive (reliability 4).

During the public consultation, more recent studies (6 studies: Lo et al., 2016, Kobayashi et al., 2013, Moermond et al., 2007, Ke et al., 2007, Lie et al., 2018, Xia et al., 2015) conducted with fish were transmitted. The BCF values reported vary between the species, ranging from 688 L/kg for Rainbow trout (Lo et al., 2016) to 2000 L/Kg for the benthic fish *Pseudopleuronected yokohamae* (Kobayashi et al, 2013. [It is noted that the reliability of these studies has not been evaluated in detailed by the dossier submitter as the conclusion on the vB criteria is not based on fish data. However, this information is added for information.]

Contrary to fish, the highest BCF values are reported specifically for molluscs. The support document for identification of CTPHT as SVHC (ECHA, 2009) summarised the studies as follows:

Bruner et al. (1994) exposed the zebra mussel Dreissena polymorpha in a static system to 3H-labelled benzo(a)pyrene and pyrene were. *The BCF calculated using kinetic rate constants ranged from 13 000 to 35 000 L/kg for pyrene.* A reliability of 2 (valid with restriction) was attributed (ECHA, 2009). Another study conducted also with *Dreissena polymorpha* is reported by Gossiaux *et al*., 1996. *In the study, organisms were exposed in a static system to radiolabelled benzo(a)pyrene in combination with pyrene. In total a number of 23 experiments with benzo(a)pyrene and 10 experiments with pyrene were conducted under either ambient field temperatures or laboratory temperatures. BCFs were calculated using kinetic rate constants and ranged from 37 000 to 43 000 for pyrene* (reliability of 2, ECHA 2009).

McLeese and Burridge (1987) determined PAH accumulation in the clam Mya renaria, the mussel Mytilus edulis, the shrimp Crangon septemspinosa and the polychaete worm Nereis virens. Groups of the invertebrates were exposed for 4 days in seawater containing a mixture of five PAHs (phenanthrene, fluoranthene, pyrene, triphenylene, and perylene) in continuous flow-systems. After 4 days, exposure was terminated, and the animals were maintained in flowing seawater at 10 °C for two weeks. Measured concentrations in water and animals were used to calculate ku and ke, which were subsequently used to calculate BCFs. For clam, mussel, shrimp and polychaete BCFs for pyrene were 6 430, 4 430, 225 and 700 L/kg respectively. A reliability of 1 (valid without restriction) was attributed to the study (ECHA, 2009).

Richardson et al. (2005) exposed the green-lipped mussel (Perna viridis) to anthracene, fluoranthene, pyrene and benzo(a)pyrene in a renewal system for 20 days followed *by a depuration period of 10 days.* The BCF calculated by kinetic approach after lipid normalisation to 5% was 44 550 L/kg (Reliability of 2, Bleeker and Verbruggen, 2009).

In addition, the support document for identification of CTPHT as SVHC (ECHA, 2009) summarised also reliable studies conducted with crustacean organisms as follows:

Bioaccumulation in Daphnia magna was studied by Newsted and Giesy (1987), the study conducted in a static system allowed to calculate a BCF at steady state of 2702 L/kg (reliability of 2, ECHA 2009). *In a study by Southworth (1978) the potential for bioaccumulation in Daphnia pulex was studied for PAHs in a static system. The bioconcentration factor was determined at steady state conditions and as the ratio between the rates of uptake and elimination at non steady state conditions. The study indicated that the PAH content of Daphnia lipid was in equilibrium with the aqueous PAH concentration. The reported BCF was 2702 for pyrene* (reliability of 2, ECHA, 2009).

Additional studies conducted with crustacean organisms have been assessed by RIVM (Bleeker and Verbruggen, 2009). The assessment included the study of Landrum *et al*., 2003 with the amphipod *Diporeia spp*. In the study organisms were exposed to a range of concentrations of ^{14}C PAHs during 28 days. The BCF values for pyrene by kinetic approach ranged from 12 300 to 36 333 L/kg for the concentrations tested (concentrations between 34 µg/L to 130.7 µg/L). A reliability of 2 was attributed for the study (Bleeker and Verbruggen, 2009). Landrum (1988), conducted experiments by flow-through systems with the amphipod *Pontoporeia hoyi* exposed to selected ^{14}C radiolabelled PAHs during 6 hours followed by 14 days of depuration phase. The experiments allowed to calculate uptake and depuration rate constants for pyrene, resulting in a BCF of 166 000 L/kg (reliability of 1, Bleeker and Verbruggen, 2009). Another reliable BCF (reliability of 2) is reported with the estuarine copepod *Eurytemora affinis* from the study of Cailleau *et al*., 2009. In the experiments, the organisms were exposed in a continuous flow–through system to dissolved PAH mixture for 86 hours. A low BCF by steady state for pyrene of 900 L/kg was determined.

RIVM (Bleeker and Verbruggen, 2009) also assessed studies conducted with insect, oligochaete and polychaeta species. In the study of Wild *et al*., 1994, BCF values were determined for the larval midge *Chironomus riparius* for a range of pH tested. The BCF ranged between 713 to 1 227 L/kg (reliability of 2, Bleeker and Verbruggen 2009). The study of Ankley *et al*., 1997, calculated a BCF of 1 720 L/kg for *Lumbriculus variegatus* after 96h of exposition to pyrene. A reliability of 2 was attributed by Bleeker and Verbruggen, 2009. However, in the experiments of Frank *et al*., 1986 with the oligochaete *Stylodrilus heringianus* a high BCF of 6 688 L/kg was reported by kinetics approach. For these studies, a reliability of 2 was attributed (Bleeker and Verbruggen, 2009).

Additional studies on bioaccumulation of pyrene were identified in the recent literature search, especially on amphipods as follows:

Carrasco-Navarro *et al*., 2015, showed the influence of temperature on the bioaccumulation, toxicokinetics, biotransformation and depuration of pyrene in the arctic marine amphipod *Gammarus setosus* at two temperatures, 2 and 8 °C. The experiments were conducted by water exposure of radiolabeled pyrene at concentrations of 1.96 ± 0.11 and 1.99 ± 0.15 µg/L for the 2 and 8°C treatments, respectively. The organisms were exposed to pyrene during 168 hours followed by 96 hours of depuration. The sampling times were 6, 24, 48, 96 and 168 h during the uptake phase and 6, 24, 48 and 96 h during the depuration phase. Analyses of the parent substance and total metabolites were conducted. Regarding the stability of the substance in water, the study mentions that the water concentrations decreased by 24 and 44% (average) after 6 and 24 h, respectively. The authors attributed the decrease to the uptake of pyrene by the organisms and to its sorption to the glass walls of the beakers. The average water concentration was used for the estimation of the toxicokinetics values. A two–compartment model was used to fix experimental values of total body burden, total metabolites and parent substance concentrations. The authors determined a BCF for the total body burdens of 13 913 L/kg (2 420 - 79 964) and 2 891 L/kg (1 540 - 5 425) for both temperature tested, respectively.

It is noted that no information is given in the paper about the physiological conditions of the organisms at the end of the experiments and whether they were fed during the experiments. It is noted also that the water concentrations were not reasonably constant over the entire exposure period. Furthermore, the BCF values reported in the paper should be treated also with caution because they were not calculated based on first order kinetics, one compartment as recommended by OCDE 305 and REACH guidelines.

Another study, Bustamante *et al*., 2012, followed the kinetics of uptake and depuration of ^{14}C-pyrene in the organs and tissues (mantle, muscle, gills, digestive gland, and the remaining soft tissues) of *Crassostrea gigas* exposed via seawater. Oysters were exposed during 24 h to ^{14}C-pyrene then placed in depuration conditions for 15 days. Forty four oysters were placed in a 50 L glass aquarium containing natural seawater (closed circuit) spiked with ^{14}C-labelled pyrene. The initial pyrene concentration in the aquarium was 0.27 µg L−1 and every 30 min seawater radioactivity was measured and pyrene was added in order to keep its concentration constant during the whole uptake phase. Oysters were not fed during the exposure period and they were collected at 0, 2, 5, 9, 19 and 24 h during the uptake and at 0, 6, 18, 36, 96, 192 and 336 h during the depuration phase. It was possible to calculate uptake and depuration rates for every organ and tissues analysed. Considering whole body a BCF of 1 560 ± 630 L/kg was reported.

It is noted that no information about the water concentrations during the time of exposition are reported in the paper. Furthermore, the authors indicated that the state of equilibrium was not reached during the time of exposition. Thus, considering these observations the BCF reported in the paper should be treated with caution.

3.3.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

The European Union Risk Assessment Report on CTPHT (EC, 2008) estimated BCF values on earthworm for several PAHs according to the equilibrium portioning approach (EP). For pyrene, it was estimated a BCF earthworm of 1 200 L/kg. This value represents a reasonable worst case (EC, 2008).

3.3.3 Field data

Bleeker and Verbruggen, 2009, reported a BAF for fish (*Acanthogobius flavimanus) of* 1 010 L/kg from the results of the study of Takeuchi *et al*. (2009).

Trophic magnification studies are available for pyrene in some food webs from different locations. In the study of Wan *et al*., 2007, PAHs concentrations in phytoplankton, zooplankton, invertebrates, fishes, and one seabird species collected from Bohai Bay on the north of China were analysed. The TMF calculated for pyrene was 0.17. In another study, a TMF of 0.74 (0.66–0.82) was calculated from the analysis of PAHs on benthic and pelagic food chain from the Baltic Sea (Nfon *et al*., 2008). The bioaccumulation and biomagnification of PAHs were investigated by Khairy *et al*., 2014, from the analysis of 11 fish species and the blue crab, *Callinectes sapidus*, collected from the fresh-brackish portion of the Passaic River. The authors calculated a TMF of 0.24 for pyrene.

The study conducted by Wang *et al*., 2012 reported estimated TMF values for PAHs, based on the analysis of several fishes with different feeding behaviors (herbivorous, omnivorous and carnivorous), collected on Taihu Lake in China. A TMF of 1.12 was calculated for pyrene, the authors suggested biomagnification of the chemical through a food chain.

It is mentioned in the chapter R.11 of the ECHA guidance on PBT or vPvB that the results from field studies should be considered as part of the overall evaluation of the bioaccumulation properties of a substance. However, currently there is no consensus about standard methodologies and guidelines for the interpretation of such results, generating uncertainties for the interpretation of those results. In particular, temporal and spatial variability or the inclusion/exclusion of a few or even a single species could affect the outcome of the TMFs. Thus, the data treatment could have a deep impact on the TMF values that were calculated. Ultimately, chapter R.11 of the ECHA guidance on PBT or vPvB indicates that the absence of a biomagnification potential cannot be used on its own to conclude that the B or vB criteria are not fulfilled.

3.3.4 Summary and discussion of bioaccumulation

The bioaccumulation potential of pyrene differs between organisms due to their capacity to metabolise (biotransform) PAHs. It is likely that pyrene is transformed in fish using enzymes belonging to the Cytochrome P450 enzymes (Cyt P450) and other mechanisms and subsequently excreted (Bleeker and Verbruggen, 2009), resulting in a low to moderate

BCF. Regarding reliable data reported with fish, the BCF values range from 97 to 1 297 L/kg.

Contrary to fish, many invertebrate species have a lower metabolic capacity, resulting in highest BCF values. Indeed, very high BCFs are reported for some species of molluscs and range from 4 430 L/kg to 77 000 L/kg. Among crustaceans, the BCF vary considerably between the species ranging from 225 L/kg for *Crangon septemspinosa* to 166 000 L/kg for the amphipod *Pontoporeia hoyi.* In addition, a high BCF of 6 688 L/kg is reported with the oligochaete *Stylodrilus heringianus*.

4. Human health hazard assessment

The evaluation of the T criteria is based on the environmental toxicity of pyrene. Human health hazards were not assessed in this dossier.

5. Environmental hazard assessment

5.1 Aquatic compartment (including sediment)

Several environmental toxicity studies on aquatic organisms have been assessed in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008) and summarised in the Support Document for identification of CTPHT as SVHC (ECHA, 2009). As the data presented in the following sections is mainly based on these documents, they will not be assessed and discussed again within this dossier. Additional relevant studies were retrieved in a bibliographic search up to June 2018 and have been included in the analysis hereafter.

The Support Document for the identification of CPHT as SVHC summarises the following on environmental hazard assessment for the aquatic compartment:

PAHs can be toxic via different modes of action, such as non-polar narcosis and phototoxicity. Phototoxicity is caused by the ability of PAHs to absorb UVA radiation, UVB radiation, and in some instances, visible light. It may occur as the result of the production of singlet oxygen, which is highly damaging to biological material, or as result of the formation of new, more toxic compounds from the photomodification (usually oxidation) of PAHs (Lampi et al., 2006). Phototoxic effects can be observed after a short period of exposure, which explains why for PAHs like anthracene, fluoranthene and pyrene, where phototoxicity is most evident, the acute toxicity values under simulated solar radiation may be lower than the chronic toxicity values determined under less harsh radiation.

The phototoxicity of PAHs is relevant where the PAHs are exposed to light and UV radiation, and considered to be most important for upper layers of aquatic and terrestrial environments. Although UV penetration depths may vary among PAH contaminated sites, it is not unlikely that significant portions of the aquatic community may be exposed to UV levels sufficient to induce phototoxicity, as UV levels occurring under normal sun light conditions have been shown to elicit these effects. There is growing evidence which suggests that phototoxic PAHs may be degrading aquatic habitats, particularly those in highly contaminated areas with shallow or clear water. Photo-induced chronic effects have been reported for anthracene at UV intensities occurring at depths of 10-12 m in Lake Michigan (Holst and Giesy, 1989). Phototoxicity of PAHs may also be initiated in aquatic organisms which have accumulated PAHs from the sediment and subsequently are exposed to sun light closer to the surface (The Netherlands, 2008).

Phototoxic effects of PAHs are therefore considered relevant in this hazard, respectively T-assessment.

5.1.1 Fish

5.1.1.1 Short-term toxicity to fish

No data are presented in the support document for identification of CTPHT as SVHC (ECHA, 2009).

Results from a short term study with fish are available in the literature. Oliveira *et al.*, 2012 conducted a standard bioassay (OECD 203) on juvenile fish of the specie *Pomatoschistus microps.* Organisms were exposed to 0.125, 0.25, 0.5 or 1 mg/L of pyrene during 96 hours. Mortality, swimming performance and several biomarkers were used as effect criteria. The study reported a LC_{50} (96 h) for mortality of 0.871 mg/L. Furthermore, a significant decrease of swimming performance of fish was observed at all the concentrations tested. The study is considered as valid (reliability of 1).

5.1.1.2 Long-term toxicity to fish

No data are available on long term toxicity to fish.

5.1.2 Aquatic invertebrates

5.1.2.1 Short-term toxicity to aquatic invertebrates

Selected short term toxicity data with pyrene are summarised in the support document for identification of CTPHT as SVHC (ECHA, 2009). The selected studies are reported in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008) as follow:

Regarding freshwater invertebrates, *the lowest value for Daphnia magna is reported in the study of Wernersson, 2003. In the experiments neonates were exposed for 24 h with 16:8 h of light:dark, then for 2 hours at an UV-intensity of 370±20 µW/cm*^{2} *(295-365 nm; peak 340 nm) and 1 hour of recovery in the test medium. The* EC_{50} *calculated for immobility was 1.38 µg/L for pyrene.*

Another study showed that when Daphnia magna neonates are exposed to UV-B radiation only (intensity 64 µW) for four times two hours during 48 hours, the EC_{50} *for immobility ranges from 2.7 to 20 µg/L at different hardness of the artificial test media and different concentrations of dissolved organic matter of natural waters (Nikkilä et al., 1999).*

Also a low acute value is reported for the fresh water mollusc Utterbackia imbecilis, the LC_{50} *(24h) was 2.63 µg/L when organisms are exposed to UV-A radiation (320-400 nm) at an intensity of 70 µW (Weinstein and Polk, 2001*).

Acute data for marine species are also available and the lowest acute values are reported in the Annex XV transitional dossier (The Netherlands, 2008) as follows.

The lowest effect concentrations are similar to fresh water species in the presence of UV-radiation for embryos/larvae of molluscs and neonates/nauplii of crustaceans.

The study of Kagan et al., 1985, reported a LC_{50} *for nauplii of Artemia salina of 8 µg/l, when organisms are exposed for 2 hours in the dark followed by one hour with UV-radiation (320-400 nm; peak 350 nm) at an intensity of 1300 µW/cm2. When exposed for 2 hours in the dark followed by eight hours with UV-radiation (peak 312 nm) at an intensity of 975-1000 µW/cm2, the* LC_{50} *for nauplii of Artemia salina was estimated from the presented figure to be 36 µg/L (Peachey and Crosby, 1996). The same treatment with sunlight (λ>290 nm) at an intensity of 407-1429 µW/cm2 resulted in an* EC_{50} *of 3.4 µg/l (Peachy and Crosby, 1996). From these results it may be concluded that the maximum intensity of the radiation is more important than the time of irradiation.*

The crustaceans Mysidopsis bahia was the most sensitive species, with a LC_{50} of 0.89 µg/l calculated in the experiment conducted by Pelletier et al., 1997. In the study, the organisms were exposed to concentrations of pyrene under ultraviolet light with an intensity of 397±35.1 µW/cm2 UV-A (365±36 nm) and 134±22.8 µW/cm2 UV-B (310±34 nm) with a photoperiod of 16:8 hour of light:dark.

Table 4: Overview of studies concerning the acute toxicity of pyrene to freshwater and marine invertebrates.
**Source: Support Document for identification of CTPHT as SVHC (ECHA, 2009)*

	Duration	Endpoint	Value	Comment	Reference
Freshwater organisms, acute tests					
Daphnia magna	27h	EC_{50} immobility	1.38 µg/L	Neonates 16:8 hours light: dark for 24 hours UV radiation for 2 hours and 1h recovery	Wernersson, 2003*
Daphnia magna	48 h	EC_{50} immobility	2.7 to 20 µg/L	Nenonates UV-B radiation	Nikkilä *et al.*, 1999*
Utterbackia imbecilis	24h	LC_{50}	2.63 µg/L	UV-A radiation	Weinstein and Polk 2001*
Marine organisms, acute tests					
Artemina salinas	3h	LC_{50}	8 µg/L	2 hours in the dark UV radiation for one hour	Kagan *et al.*, 1985, 1987*
Artemina salinas	10 h	LC_{50}	36 µg/L	2 hours in the dark followed by eight hours with UV radiation	Peachy and Crosby, 1996*
Artemina salinas	10 h	LC_{50}	3.4 µg/L	2 hours in the dark followed	Peachy and Crosby, 1996*
Mysidopsis bahia	48 h	LC_{50}	0.89 µg/L	16:8 hour light: dark UV- A B radiation	Pelletier *et al.*, 1997

5.1.2.2 Long-term toxicity to aquatic invertebrates

Chronic toxicity data are available for several aquatic invertebrates; the following studies were presented on the Support document for identification of CTPHT as SVHC (ECHA, 2009), as follows:

Chronic toxicity data are reported for fresh water species with EC_{10} values of 1.2 (algae, Bisson et al., 2000) - 2.1 µg/L (Ceriodaphnia dubia, Bisson et al., 2000) and for one marine oyster (Crassostrea) with a NOEC for shell development of 0.5 µg/l (Lyons et al., 2002).

As this latter NOEC value was the lowest one from a reliable study, it was chosen as the key study.

Further long term studies with invertebrates were assessed by Verbruggen, 2012. In the following table, the studies evaluated by Verbruggen, 2012 as the most reliable studies (reliability of 2) are also included.

Table 5: Overview of studies concerning the long term toxicity of pyrene to freshwater and marine invertebrates.

Species	Duration	Endpoint	Value	Comment	Reference
Freshwater organisms, long-term tests					
Ceriodaphnia dubia	7 d	EC_{10} reproduction	2.1 µg/L	at less than 500 lux No presence of UV radiation	Bisson *et al*., 2000
*Hyalella Azteca**	10 d	LC_{50} Mortality	77.1 µg/L	No presence of UV radiation	Lee et al., 2001
Marine organism, long-term tests					
Crassostrea gigas	48 h	NOEC shell development	0.5 µg/L	Embryos/larvae 12:12 hour light: dark UV A B radiation	Lyons *et al*., 2002
*Arcatia tonsa**	72 h	EC_{10} recruitment	1.7 µg/L	UV light with an intensity of 8µW/cm^2 (UV-A) and 11.9 µW/cm^2 (UV-B)	Bellas and Thor 2007
*Mytilus galloprovincialis**	48 h	EC_{10} larval development	8.3 µg/L	Photoperiod 14:10 hours light: dark by cool daylight lapms (280-780 nm, PAR) with an intensity of 70 µE/m^2/s	Bellas et al. 2008
*Paracentrotus lividus**	48 h	EC_{10} larval development	23 µg/L	Photoperiod 14:10 hours light: dark by cool daylight lapms (280-780 nm, PAR) with an intensity of 70 µE/m^2/s	Bellas et al. 2008

Mulinea lateralis	48h	LC_{50} Survival/ development	0.23 µg/L	UV light with 397 µW/cm^2 (UV-A) and 134 µW/cm^2 (UV-B) with photoperiod 16:8	Pelletier et al., 1997

Source: Support Document for identification of CTPHT as SVHC (ECHA, 2009), *From Verbruggen, 2012

5.1.3 Algae and aquatic plants

For algae and aquatic plants, one study was considered in the Support Document for identification of CTPHT as SVHC (ECHA, 2009).

Bisson et al., 2000, reported an EC_{10} value of 1.2 µg/L from a 72-h static study with Pseudokirchneriella subcapitata.

Table 6: Overview of the algae test with pyrene.

Species	Duration	Endpoint	Value	Comment	Reference
Algae, long term test					
Pseudokirchneriella subcapitata	72h	EC_{10} growth	1.2 µg/L	Light intensity 6000-8000 lux No presence of UV radiation	Bisson *et al.*, 2000
*Scenedesmus vacuolatus**	24h	NOEC	21 µg/L	No presence of UV radiation	Altenburger et al., 2004

*Source: Support Document for identification of CTPHT as SVHC (ECHA, 2009), * From Verbruggen, 2012*

5.1.4 Sediment organisms

Several toxicity studies of pyrene with living sediment organisms were assessed and reported in the Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008). Furthermore, those studies were reassessed later by RIVM (Verbruggen, 2012), the results were reported as follows:

Toxicity studies with 2 freshwater oligochaetes are available. The lowest value for Limnodrilus hoffmeisteri is the EC_{10} from a 28-d reproduction study (Lotufo and Fleeger, 1996). However, this value was extrapolated from concentrations showing more than 40% effect. Therefore, the EC_{10} of 3.8 mg/kgdw (32 mg/kgdw, standard soil, recalculated to standard sediment with 10% organic matter) has a large uncertainty. Further, the reproduction falls rapidly with concentrations up to 210 mg/kgdw (1770 mg/kgdw, standard soil), but remains almost constant from 210 to 841 mg/kgdw (1770-7070 mg/kgdw, standard soil). It is plausible that the bioavailability of pyrene in the sediment is limited at the higher concentrations by the solubility in the pore water of sediment. Possibly, pore water concentrations are already saturated around 400 mg/kgdw, standard *soil. If the 2 highest concentrations are omitted from the determination of EC_{10}, the resulting value of 26 mg/kgdw (220 mg/kgdw, standard soil) in standard sediment is much higher. Further, the reported EC_{25} values determined by bootstrapping, are not in accordance with the log-logistic fit by which the EC_{10} is derived. The EC_{25} values reported by Lotufo and Fleeger (1996) for sediment egestion from a 10-d and a 5-d study and for reproduction from a 28-d study are 51.6, 58.9, and 59.1, respectively, for a sediment with 1.2% organic carbon. With the derived EC_{50}, which is rather certain because it is not an extrapolated value and the reported EC_{25}, EC_{10s} can be derived with a log-logistic model. For the endpoints mentioned above, these EC_{10} values recalculated to sediment with 10% organic carbon are 222, 217, and 255 mg/kgdw, respectively. These values probably are more realistic.*

[…]
Some 10-d experiments with Rhepoxynius abronius were performed (Swartz et al., 1997). The LC_{10s} derived from the presented data are 45 and 154 mg/kgdw standard sediment, the confidence limits of these values are rather small.

Table 7: Overview of the studies with sediments organisms

Species	Duration	Endpoint	Value	Comment	Reference
Sediment organisms					
Limnodrilus hoffmeisteri	10 d - 5 d	EC_{10} Sediment egestion	222-217 mg/kg_{dw}		Lotufo and Fleeger, 1996 Verbruggen, 2012
Rhepoxynius abronius	10 d	LC_{10} Mortality	45-154 mg/kgd_{dw}	After 10 days, survivals were exposed to UV radiation for 1 h	Swartz *et al.*, 1997

5.2 Terrestrial compartment

Several long term toxicity data are available regarding terrestrial organisms. The Annex XV Transitional Dossier for CTPHT (The Netherlands, 2008) presents the results of the following studies.

The lowest value is the 28-day NOEC for reproduction of Folsomia candida reported by Bleeker et al., 2003. The NOEC recalculated to soil with an organic carbon content of 2% is 10 mg/kg_{dw}.

The EC_{10}/NOEC for reproduction Folsomia fimetaria and F. candida are very similar to this value, varying from 10 to 21 mg/kg_{dw} recalculated to a soil with 2% organic matter. Ageing of the compound for a period 120 days had little effect on the toxicity (Sverdrup et al., 2002c).

Experiments conducted with the annelid Enchytraeus crypticus shows from a 21-day reproduction study (Sverdrup et al. 2002b) a NOEC of 23 mg/kg_{dw} for standard soil (EC_{10} of 14 mg/kg_{dw}). A similar 28-day reproduction study by Bleeker et al. (2002) results in a NOEC of 160 mg/kg_{dw} for standard soil. The NOEC for growth of Eisenia veneta from a 28-day study (Sverdrup et al., 2002d) was 37 mg/kg_{dw}, recalculated to 2% organic carbon (EC_{10} of 48 mg/kg_{dw}). Droge et al. (2006) reported an EC_{50} value of 52 mg/kg_{dw}.

Regarding terrestrial plants, the study conducted by Sverdrup et al., 2003, shows the fresh weight as the most sensitive endpoint in the experiments with Sinapsis alba, Trifolium pratense and Lolium perenne, with an exposure time of 19 to 21 days. From the reported EC_{50} *and* EC_{20} *values,* EC_{10s} *can be derived with a log-logistic relationship. These* EC_{10} *values for the three terrestrial plants are 34, 19, and 546* mg/kg_{dw} *respectively for a soil with 2% organic carbon.*

Finally, for nitrification, NOEC and EC_{10} *values of 99 and 160* mg/kg_{dw} *(recalculated to a soil with 2% organic carbon) are reported from the study of Sverdrup et al., 2002a.*

5.3 Summary and discussion of the environmental hazard assessment

Several short-term toxicity studies are available with freshwater and marine organisms. The lowest EC_{50} values were those reported in *Daphnia magna* studies (1.38 µg/L and 2.7 µg/L) in presence of UV radiation. Concerning marine organisms, the lowest acute effect concentrations were reported for *Mysidopsis bahia* (0.89 µg/L) also in presence of UV radiation.

Regarding long-term toxicity, data are reported for two fresh water trophic levels (algae and crustacea) with EC_{10} values of 1.2-2.1 µg/L and for one marine organism (mollusc) with a NOEC for shell development of 0.5 µg/L. This last study was chosen as key study for the assessment of T properties (ECHA, 2009).

6. Conclusions on the SVHC Properties

6.1 CMR assessment

No harmonised classification relevant for the PBT assessment is available for pyrene. The substance is identified in this report in accordance with Article 57(d) whereas for the toxicity only ecotoxicity is considered in the assessment. For Article 57(e) assessment of toxicity is not relevant.

6.2 PBT and vPvB assessment

An assessment of the PBT/vPvB properties of pyrene has been carried out by the MSC in the scope of the identification of CTPHT as SVHC. The PBT/vPvB assessment of this dossier is based on information provided in the support document for the identification of CTPHT as SVHC (ECHA, 2009).

Earlier assessments documented in the EU Risk Assessment Report on CTPHT (European Commission, 2008) and the Annex XV Transitional Dossier on CTPHT (The Netherlands, 2008) have been considered as additional information. Furthermore, additional relevant data retrieved from a literature search until June 2018 and from public consultation were incorporated.

6.2.1 Assessment of PBT/vPvB properties

6.2.1.1 Persistence

Pyrene has a low water solubility and shows a high tendency to adsorb to particles and organic matter in the environment. The resulting low bioavailability is one of the limiting factors of its biodegradation.

Experimental data shows that pyrene exhibits a low biodegradation in the soil compartment. The study conducted under microcosm conditions by Wild and Jones (1993) reported half-life values of 127 to 320 days, and the experimental study in field conditions (Wild *et al*., 1991) supports the very low degradation of pyrene in soils, the authors demonstrated a half-life of 8.5 years. A study of Harmsen and Rietra, 2018 suggests that degradation rates of pyrene may depend on the initial concentration and that a certain fraction can remain for a long period of time in the environment

Furthermore, a very low degradation rate of pyrene is also expected for the sediment compartment under anaerobic conditions. In view of the fact that phenanthrene meets the P and vP criterion in a sediment simulation study (Meisterjahn et al. 2018), it is assumed that pyrene will meet P and vP criterion as well considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in sediment are proportionally related to the octanol-water partition coefficient (Kow) (Durant et al. (1995) cited in The Netherlands, 2008).

Model calculations done by Mackay *et al*. (1992) support the high half-life values measured in the previous studies cited.

Therefore, it is concluded that pyrene fulfils the P and vP criteria for soil and sediment according to REACH Annex XIII.

6.2.1.2 Bioaccumulation

Reliable experimental BCF values higher than 5 000 are reported for some species of molluscs (6 430 to 77 000 L/kg), crustacean (12 300 to 166 000 L/kg) and one species of oligochaete *(*6 688 L/kg).

Considering that all the reliable BCF values measured are above the B and vB criteria according to REACH Annex XIII, it is concluded that pyrene is a bioaccumulative and very bioaccumulative substance.

This conclusion was already drawn in the Support Document for identification of CTPHT as SVHC (ECHA, 2009). New data retrieved in the literature search done in June 2018 have been included in the current report and did not challenge the previous conclusion.

6.2.1.3 Toxicity

Regarding long-term results, the highest toxicity of pyrene for aquatic organisms observed has been in the mollusc Crassostrea *gigas* under exposition of UV radiation and the EC_{10} value reported for shell development was 0.5 µg/L (Lyons *et al*. 2002).

Furthermore, EC_{10} values lower than 0.01 mg/L from long-term studies have been also reported for pyrene for the algae *Pseudokirchneriella subcapitata* in the absence of UV radiation (EC_{10} growth inhibition of 1.2 µg/L), for *Arcatia tonsa* under exposition of UV radiation (EC_{10} recruitment of 1.7 µg/L) (Bellas and Thor 2007), for *Ceriodaphnia dubia* in absence of UV radiation (EC_{10} reproduction of 2.1 µg/L) (Bisson *et al*., 2000) and *Mitilus galloprovincialis* (EC_{10} larval development of 8.3 µg/L) (Bellas et al., 2008).

Based on the available experimental aquatic long-term data, pyrene fulfils the T criteria according to REACH Annex XIII (NOEC/EC_{10} for aquatic organisms less than 0.01 mg/L).

This conclusion was already drawn by the MSC in the context of the identification of CTPHT as SVHC (ECHA, 2009). New data retrieved in the literature search done in June 2018 have been included in the current report and did not challenge the previous conclusion.

6.2.2 Summary and overall conclusions on the PBT and vPvB properties

The assessment of the PBT/vPvB properties in the present dossier and the conclusion that pyrene fulfils the criteria in Article 57 (d) and (e) were based mainly on the information in the MSC Support Document on CTPHT (ECHA, 2009) and supplemented with information from newer studies. This information was considered together in a weight-of-evidence approach.

Persistence

The available experimental information shows that pyrene degrades very slowly in soils exhibiting half-lives between 127 to 320 days, higher than 180 days (vP criteria according REACH Annex XIII). The soil experimental study conducted under field conditions and the predicted half-life support the low degradation in soils.

It is also assumed that pyrene meets the P and vP criterion in sediment, as in the available simulation study with phenanthrene the half-life meets the P and vP criterion. Considering that the biodegradation rates decrease with increasing number of aromatic rings and the half-lives of PAHs in sediment are proportionally related to the octanol-water partition coefficient (Kow), the half-life of pyrene should meet the P and vP criteria in sediment as well.

Thus, pyrene fulfills the criteria for P and vP of REACH Annex XIII for soil and sediment.

Bioaccumulation

BCF values higher than 5 000 (vB criteria according REACH Annex XIII) have been measured in four studies with molluscs (6 430 to 77 000 L/kg), two studies with crustacean (12 300 to 166 000 L/kg) and one study with an oligochaete species (6 688 L/kg).

Thus, pyrene fulfils the B and vB criteria of REACH Annex XIII.

Toxicity

Based on the available information from long-term studies with aquatic organisms, the lowest EC_{10} value was reported for the mollusc *Crassostrea gigas* exposed to UV radiation. The resulting EC_{10} of 0.5 µg/L shows that pyrene fulfils the T criteria (NOEC or EC_{10} for marine or freshwater organisms is less than 0.01 mg/L) of REACH Annex XIII.

Overall conclusion

In conclusion, pyrene meets the criteria for a PBT and vPvB substance according to Article 57 (d) and (e) of REACH Regulation by comparing all relevant and available information according to Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

6.3 Assessment under Article 57(f)

This section is not relevant for the identification of the substance as SVHC in accordance with Article 57 points (d) and (e) of REACH Regulation.

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