Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

dibenzo[def,p]chrysene

EC Number: 205-886-4
CAS Number: 191-30-0

CLH-O-0000001412-86-243/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name:

Dibenzo[def,p]chrysene

EC Number: **205-886-4**

CAS Number: **191-30-0**

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Dibenzo[def,p]chrysene
Synonyms	Dibenzo(a,l)pyrene Naphtho[1,2,3,4-pqr]tetraphene
EC number:	205-886-4
CAS number:	191-30-0
Annex VI Index number:	-
Degree of purity:	n/a
Impurities:	n/a

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Muta. 2; H341 Carc. 1B; H350; SCL = 0.001 %
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Muta. 2; H341 Carc. 1B; H350; SCL = 0.001 %

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.	Explosives	None		None	Not addressed
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral				
	Acute toxicity - dermal				
	Acute toxicity - inhalation				
3.2.	Skin corrosion / irritation				

3.3.	Serious eye damage / eye irritation	None	None	None	Not addressed
3.4.	Respiratory sensitisation				
3.4.	Skin sensitisation				
3.5.	Germ cell mutagenicity	**Muta 2**	**None**	**None**	
3.6.	Carcinogenicity	**Carc.1B**	**SCL = 0.001 %**	**None**	
3.7.	Reproductive toxicity	None	None	None	Data lacking
3.8.	Specific target organ toxicity –single exposure				Not addressed
3.9.	Specific target organ toxicity – repeated exposure				
3.10.	Aspiration hazard				
4.1.	Hazardous to the aquatic environment				
5.1.	Hazardous to the ozone layer				

Table 4: Proposed labelling based according to the CLP Regulation

	Labelling	**Wording**
Pictograms	GHS08	Health hazard
Signal Word	Dgr	Danger
Hazard statements	H341 H350	 May cause cancer

Proposed notes assigned to an entry: -

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

This CLH proposal aims to classify and label of dibenzo[def,p]chrysene (naphtho(1,2,3,4-pqr)tetraphene; dibenzo(a,l)pyrene; DB[a,l])P) for mutagenicity and carcinogenicity. DB[a,l]P is not listed in Annex VI to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Dangerous Substances (CLP).

So far, DB[a,l]P was not listed in any priority list of the Existing Substance Regulation (Regulation 793/93/EC) and it was not previously discussed by the Technical Committee and Labelling (TC C&L) according to Council Directive 67/548/EEC.

The hexacyclic hydrocarbon DB[a,l]P belongs to the group of polycyclic aromatic hydrocarbon (PAH). PAH constitute a large class of compounds, and hundreds of individual substances may be released during incomplete combustion or pyrolysis of organic matter, an important source of human exposure. Studies of various environmentally relevant matrices, such as coal combustion effluents, motor vehicle exhaust, used motor lubricating oil and tobacco smoke have shown that the PAH in these mixtures are mainly responsible for their carcinogenic potential. However, only a small number of PAH are classified according to CLP up to and including 2015 as mutagenic category 1B (benzo[a]pyrene (B[a]P)), mutagenic Category 2 (chrysene (CHR)) or as carcinogen Category 1B (B[a]P, benzo[e]pyrene (B[e]P), benz[a]antracene (B[a]A), CHR, benzo[b]fluoranthene (B[b]F), benzo[j]fluoranthene (B[j]F), benzo[k]fluoranthene (B[k]F) and dibenz[a,h]antracene (DB[a,h]A)). In addition, B[a]P is a classified reprotoxicant which is classified as Repr. 1B. Lack of classification for the other congeners as CMR does not necessarily reflect absence of corresponding toxic effects.

The Committee for Risk Assessment (RAC) agreed at its 41st meeting (June 2017) to the proposal by Germany to classify/labelling the structurally similar substances to DB[a,l]P benzo[rst]pentaphene (DB[a,i]P) (CAS: 189-55-9) and dibenzo[b,def]chrysene (DB[a,h]P) (CAS: 189-64-0) as Muta. 2; H341 and Carc. 1B; H350.

In this dossier, the experimental data of DB[a,l]P were evaluated and a classification as mutagen and carcinogen according CLP is proposed.

DB[a,l]P is evaluated as mutagen by international bodies (SCF 2002; FAO/WHO 2006; EFSA 2008; IARC 2010) and by an international regulation program (IPCS 1998) based on the results both of in vitro testing (bacteria; proliferating cells of cell cultures) and in vivo testing (soma cells). These mutagenicity/genotoxicity tests are re-evaluated for the justification of classification as mutagen according to CLP. In addition, the genotoxicity tests are evaluated which were published after the last official statement to classification of DB[a,l]P (IARC 2010).

The carcinogenic potential of DB[a,l]P was first evaluated in December 1972 by a Working Group of IARC that restricted its evaluation to work published after 1966, since earlier data reported for DB[a,l]P had in fact been obtained from experiments. A single study was analysed that showed the induction of sarcomas following subcutaneous administration of DB[a,l]P in mice (IARC 1973).
In February 1983 a Working Group of IARC re-assessed the same bioassay and an additional study of dermal application to mice that resulted in the induction of skin tumours at the sites of application. DB[a,l]P has been found to be carcinogenic to experimental animals. On the basis of these data, the Working Group concluded that there was sufficient evidence that DB[a,l]P was carcinogenic to experimental animals (IARC 1983).

In a subsequent evaluation by IARC in 2006 additional bioassays were included. These were studies with single and repeated dermal application to mice, as well as a number of several initiation–promotion studies on mouse skin; all studies gave positive results. DB[a,l]P also induced oral squamous cell carcinoma when repeated applied dermally to the tongue of hamsters. Benign and malignant tumours in various organs, i.e. ovaries, lymphoid tissues and skin were also observed following repeated oral application by gavage of low doses DB[a,l]P to mice. Lung tumours were observed in adult mice following intraperitoneal injection. In new-born mice, DB[a,l]P induced lung and liver tumours and a variety of tumours at other sites when administered intraperitoneally. Two further studies of administration in the mammary gland of rats also yielded positive results. Based on the sufficient evidence in animals and strong mechanistic data DB[a,l]P was classified as probably carcinogenic to humans beings (group 2A) by IARC (Straif et al. 2005; IARC 2006).

The same experimental studies on DB[a,l]P are now re-evaluated for classification as carcinogen according to CLP.

2.2 Short summary of the scientific justification for the CLH proposal

A classification of DB[a,l]P as mutagen and carcinogen is proposed.

Mutagenicity

A variety of positive genotoxicity tests both in vitro and in vivo are available. An evaluation of these tests taking into account the quality of the test performances leads to the conclusion that only two positive in vitro mutagenicity tests (bacterial gene mutation test; gene mutation test at TK locus) were carried out in accordance with the corresponding EU/OECD test guideline. Due to the lack of a positive control all other tests with a positive result have a crucial methodological shortcoming. Therefore, merely the guideline compliant positive in vitro gene mutation tests are to be considered for justification of classification of DB[a,l]P as mutagen, although the other positive results from in vitro/in vivo testing seem to be conclusive.

A supporting criterion for justification of classification of DB[a,l]P as a mutagen is a read-across approach with B[a]P and CHR which have been already classified as germ cell mutagens. Due to chemical structures and the resulting metabolic activations of the substances a read-across is sufficiently justified. The read-across approach clearly supports the relevance of the positive genotoxic effects in vitro and in vivo in soma cells induced by DB[a,l]P despite the lack of positive controls in the most positive genotoxicity tests.

Due to the currently available guideline compliant positive in vitro gene mutation tests with DB[a,l]P and the read-across approach to B[a]P and CHR a classification of DB[a,l]P as a Muta. Cat. 2, H341 is proposed in accordance with CLP.

Carcinogenicity

There is sufficient evidence on carcinogenicity of DB[a,l]P by all routes tested in three rodent species (mice, rats and hamster). The available experiments have demonstrated that DB[a,l]P causes tumours at several sites, by several routes of administration, in both sexes, and in several animals species and strains under different protocols.

Repeated oral application of low dose of DB[a,l]P by gavage to mice of two strains induced benign and malignant tumours in various organs, e.g. ovaries, uterus, lymphoid tissues and skin; and oral squamous cell carcinoma after repeated applications of low doses on the tongue of hamsters.

Dermal exposure (by single and repeated topical administration) to low doses of DB[a,l]P caused benign or malignant skin tumours (squamous cell papilloma or squamous cell carcinoma) in mice, and single subcutaneous injection caused cancer at the injection site (sarcoma) in mice. Single intra-peritoneal injection of mice with DB[a,l]P caused benign and malignant lung tumours, and repeated injections of new-born mice lung adenomas and benign and malignant liver tumours in both sexes. DB[a,l]P administered in the mammary gland by a single injection caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in female rats. In dermal initiation-promotion studies the tumour initiating activity of DB[a,l]P to the skin was demonstrated in several mouse strains.

No species-specific mode of action for DB[a,l]P carcinogenesis was identified.

DB[a,l]P is possibly carcinogenic to humans. Classification as carcinogen is largely based on animal evidence. DB[a,l]P meets the criteria for classification and labelling as Category 1B carcinogen, H350 according to CLP.

2.3 Current harmonised classification and labelling

DB[a,h]P is not classified according Annex I to CLP Regulation.

2.4 Current self-classification and labelling

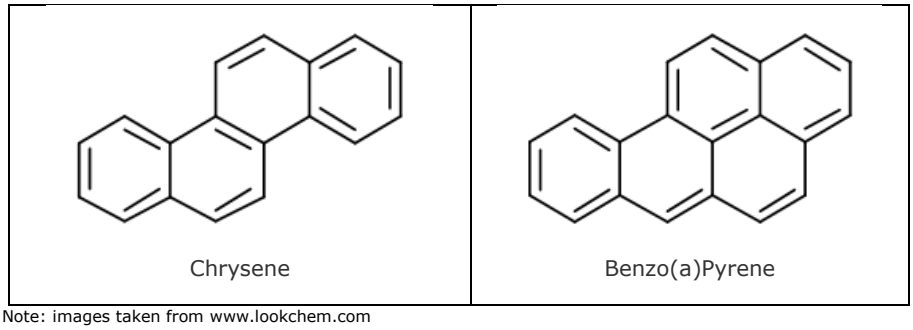
Table 5: Entry in the C&L inventory to dibenzo[def,p]chrysene (October 2017)

Classification		Labelling			Specific Concentration limits, M-Factors	Number of Notifiers
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Supplementary Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**		
Eye Dam. 1 Carc. 1B	H318 H350	H318 H350		GHS05 GHS08 Dgr		23
Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H350 H400 H410	H350 H400 H410		GHS09 GHS08 Dgr		4
Not classified						3

Number of aggregated notifications: 3

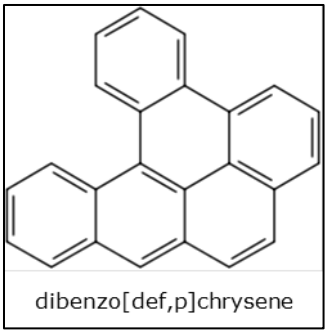
RAC general comment
Only two endpoints were presented in the Dossier Submitter's (DS) proposal for harmonised classification and labelling of dibenzo[def,p]chrysene: germ cell mutagenicity and carcinogenicity. Although data from laboratory tests with this substance were presented for both endpoints, a key supporting aspect of the proposal is its structural and biochemical similarity to polycyclic aromatic hydrocarbons (PAHs) that are well known to possess these hazards.

There are more than 100 substances that can be identified as PAHs. They are commonly formed by the incomplete combustion of organic substances, including the burning of wood, coal and tobacco. The main structural characteristics of PAHs are that they are generally planar, highly conjugated aromatic compounds. In their report, the DS presented data on two PAHs that have been established as having carcinogenic and mutagenic potential: chrysene (4-membered benzene ring structure) and benzo(a)pyrene (B[a]P) (5-membered ring structure).

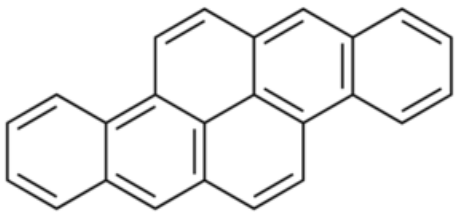


Note: images taken from www.lookchem.com

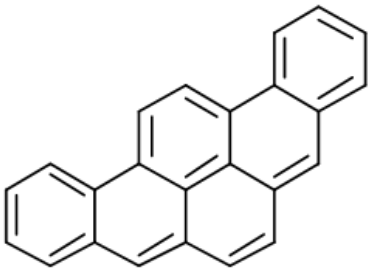
As can be seen from the following diagram, dibenzo[def,p]chrysene is a larger molecule, with 6 rings, but still has structurally similarity to both chrysene and B[a]P.



Although details are not included in the CLH report for this substance, the DS previously submitted classification proposals for 2 other PAHs: dibenzo[b,def]chrysene and benzo[rst]pentaphene. RAC agreed that these should both be classified for mutagenicity and carcinogenicity based on relevant toxicological studies and a comparison with chrysene and B[a]P. Both of dibenzo[b,def]chrysene and benzo[rst]pentaphene have a 6 ring structure.



Dibenzo[b,def]chrysene



Benzo[rst]pentaphene

The subject of this opinion, dibenzo[def,p]chrysene, differs from the others in that it has both the classical "bay region" sub-structure that is common to all these PAHs and a deeper "fjord region". These are reactive electrophilic regions that have potential to bind with nucleophilic sites in macromolecules such as DNA, RNA and proteins. The adenine and guanine bases in single or double stranded DNA are sensitive targets and binding at these sites has been shown to cause mutations that have been implicated in the carcinogenicity of PAHs.

Given the structural similarities of B[a]P, chrysene and dibenzo[def,p]chrysene, the DS used this information in conjunction with available data to classify dibenzo[def,p]chrysene for germ cell mutagenicity and carcinogenicity.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

DB[a,h]P has CMR properties that justify a harmonised classification and labelling according to article 36 of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

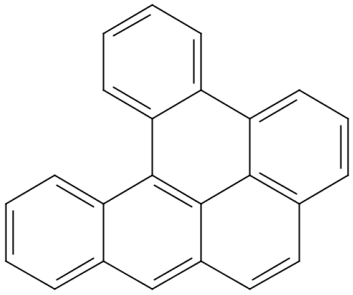
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 6: Substance identity

EC number:	205-886-4
EC name:	Dibenzo[def,p]chrysene
CAS number (EC inventory):	191-30-0
CAS number:	191-30-0
CAS name:	Dibenzo[def, p]chrysene
IUPAC name:	Dibenzo[def,p]chrysene
Public names:	Dibenzo[def,p]chrysene Dibenzo(a,l)pyrene Naphtho[1,2,3,4-pqr]tetraphene
CLP Annex VI Index number:	-
Molecular formula:	$C_{24}H_{14}$
Molecular weight range:	302.37 g/mol

Structural formula:



1.2 Composition of the substance

No registration data are available for the substance dibenzo[def,p]chrysene (dibenzo(a,l)pyrene, status: October 2017).

Table 7: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Dibenzo[def,p]chrysene			

Current Annex VI entry: -

Table 8: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

Table 9: Additives (non-confidential information)

Additive	Function	Typical concentra-tion	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

The term polycyclic aromatic hydrocarbon (PAH) commonly refers to a large class of organic compounds that contain only carbon and hydrogen and are comprised of two or more fused aromatic rings. DB[a,l]P is a hexacyclic polynuclear aromatic hydrocarbon.

Following five properties in particular have a decisive influence on the biological activity and on the toxicokinetics of PAH: vapour pressure, adsorption on surfaces of solid carrier particles, absorption

into liquid carriers, lipid aqueous partition coefficient in tissues, and limits of solubility in the lipid and aqueous phases of tissues.

Table 10: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Solid, pale yellow	IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 32, p.343-347, 1983. (secondary source)	
Melting/freezing point	162.4 °C	IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 32, p.343-347, 1983. (secondary source)	
Boiling point	595°C	PAH Position Paper Annexes, Working Group On Polycyclic Aromatic Hydrocarbons, 2001. (secondary source)	
Relative density	n.a.		
Vapour pressure	4.8*10-10 mm Hg (T = 25 °C)	SRC PhysProp Database, 2012 (secondary source)	estimated data
Surface tension	n.a.		
Water solubility	0.00036 mg/L (T = 25 °C)	SRC PhysProp Database, 2012 (secondary source)	estimated data
Partition coefficient n-octanol/water	Log Pow = 7.71 (T = 25 °C)	SRC PhysProp Database, 2012 (secondary source)	experimental data
Flash point			
Flammability			
Explosive properties			
Self-ignition temperature			
Oxidising properties			
Granulometry	n.a.		
Stability in organic solvents and identity of relevant degradation products	n.a.		
Dissociation constant	n.a.		
Viscosity	n.a.		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

PAH are contained in certain elastomer/rubber materials, and potentially also in plastic materials, lacquers/varnishes, or coatings that may be encountered in or part of consumer products, including toys. Numerous examples of such products include e.g., tool handles, bicycle handlebars, slippers, flip-flops, beach sandals, diver equipment, toy car tyres, or clay pigeons used in skeet shooting. PAH may also be contained in synthetic turf or in materials used for construction work, e.g. flooring material. During recent years, a number of laboratories have frequently identified high PAH contamination levels including DB[a,l]P in a significant fraction of analysed consumer articles, such as toys, tool handles, bicycle grips, shoes, sports equipment etc. (BfR 2009; UBA 2010; Wennemer 2009).

In analysis of consumer products for their PAH contents Hutzler et al. (2011) have identified four isomeric dibenzopyrenes (DB[a,l]P; DB[a,e]P; DB[a,i]P; DB[a,h]P). The sample with the highest dibenzopyrene content (hammer grip) was presented with 7.1 mg/kg for DB[a,i]P, 5.6 mg/kg for DB[a,h]P and 2.1 mg/kg for DB[a,l]P.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Hazard classes not assessed in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The general principles of the kinetics of PAH, in particular B[a]P, have been covered exhaustively in the published literature. Data on individual PAH are sparse. Extensive descriptions for PAH are available in the standard reviews, e.g. ATSDR (1995), IARC (1983, 2010), IPCS (1998), EFSA (2008) and WHO (1998, 2003). In this chapter, only a very brief summary is given.

Since experimental data regarding absorption, distribution, metabolism and excretion for pure DB[a,l]P are not available a general overview of the toxicokinetics of PAH is given in Section '4.1.3 Summary and discussion on toxicokinetics'.

4.1.1 Non-human information

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

Absorption

PAH are easily absorbed through the epithelia of the respiratory and gastrointestinal tract, and the skin. The absorption rate is strongly affected by various factors, such as the anatomical site, the composition of the vehicle of administration, the molecular weight of the single PAH and the dose applied. PAH adsorbed onto particulate matter are cleared from the lungs more slowly than free hydrocarbons. Absorption from the gastrointestinal tract occurs rapidly in rodents, but metabolites return to the intestine via biliary excretion. Data from both human and animal studies clearly have shown that PAH penetrate the skin and reach the systemic circulation.

In the 'Annex XV restriction report for the 8 PAH (2010)', p. 50[1] absorption rates of 50 % or 20 % following dermal application were derived. In this paper it has been concluded that the database in animals demonstrated considerable absorption across all possible routes of exposure, and the following assumptions were made: - In the absence of any substantial evidence to the contrary, absorption rates were seen as being basically comparable for all routes in these experiments, i. e. all were estimated to be in the range of about 50 %; - While this holds true for dermal absorption of BaP out of an acetone matrix, absorption from sweat was assumed to be lower by a factor of 2.5, i. e. in the order of 20 %.

[1] http://www.bfr.bund.de/cm/343/pak_annex_XV_restriction_report_proposal_for_a_restriction.pdf;

http://echa.europa.eu/addressing-chemicals-of-concern/restrictions/substances-restricted-under-reach/-/dislist/details/0b0236e1807e2cba

Distribution

When absorbed, PAH are distributed via the bloodstream throughout all internal organs, and particularly in those with high fat contents. Intravenously injected PAH are cleared rapidly from the bloodstream of rodents but can cross the placental barrier and have been detected in foetal tissues.

Metabolism

The metabolism of PAH is complex. It starts at the moment PAH are absorbed through the epithelia of the lungs and the skin. In general, the process involves epoxidation of double bonds, a reaction catalysed by the cytochrome P450-dependent monooxygenase system, the re-arrangement or hydration of such epoxides to yield phenols or diols, respectively, and the conjugation of the hydroxylated derivatives. Most PAH metabolised in this way are deactivated. However, PAH may also be activated to DNA-binding species, such as diol epoxides and radical cations that can initiate cancer.

PAH-induced carcinogenesis involves a number of steps including: the enzymatic activation of the PAH metabolites; the covalent binding of PAH metabolites to DNA; and the induction of mutations that serve to initiate the transformation process as a result of PAH-DNA adducts.

Excretion

PAH metabolites and their conjugates do not persist in the body. PAH and their metabolites are rapidly excreted predominantly via the faeces and to a lesser extent in the urine. Conjugates excreted in the bile can be hydrolysed by enzymes of the gut flora and reabsorbed. This excludes those PAH moieties that become covalently bound to tissue constituents, in particular nucleic acids, and are not removed by repair.

4.2 Acute toxicity

Hazard class not assessed in this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Hazard class not assessed in this dossier.

4.4 Irritation

4.4.1 Skin irritation

Hazard class not assessed in this dossier.

4.4.2 Eye irritation

Hazard class not assessed in this dossier.

4.4.3 Respiratory tract irritation

Hazard class not assessed in this dossier.

4.5 Corrosivity

Hazard class not assessed in this dossier.

4.6 Sensitisation

4.6.1 Skin sensitisation

Hazard class not assessed in this dossier.

4.6.2 Respiratory sensitisation

Hazard class not assessed in this dossier.

4.7 Repeated dose toxicity

Hazard class not assessed in this dossier.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Hazard class not assessed in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 11: Gene mutations in bacteria

Test	Bacterial strain (S. typhi-murium tester strains)	Concentration	Metabol. activa-tion	Result and Remarks	Reference
Bacterial gene muta-tion test Similar to OECD TG 471 Crucial deficiency: no data are available for the used positive and negative controls GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	TA 98 TA 100	up to 100 nM/plate	+/-	Result: **positive** Supporting study Effect: - positive only with metabolic activation in both tested strains up to 100 nM/plate Cytotoxicity: - no information Controls: - no data are available for the used positive and negative controls Remarks: - preincubation method - lack of detailed experimental data - only graphical presentation	Devanesan et al. 1990

Test	Bacterial strain (S. typhi-murium tester strains)	Concentration	Metabol. activa-tion	Result and Remarks	Reference
Bacterial gene muta-tion test (forward muta-tion) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	TM 677	up to 30 µg/mL	+/-	Result: **positive** Supporting study Effect: - positive only with metabolic activation in a dose-depend-ent manner up to 30 µg/mL Cytotoxicity: - survival plateauing of approximately 50 % over the concentration range of 3.0 - 30 µg/mL Remarks: - as negative control only the historical negative control was taken into account - lack of detailed experimental data - only graphical representation	Busby et al. 1995
Bacterial gene muta-tion test Compliant to OECD TG 471 GLP: no information **Test substance: DB[a,l]P** Purity: no information	TA 98 TA 100 TA 104	0.1 – 5.0 µg/plate	+	**Key study** (reliable study; OECD TG 471 with deviations (see below)) Result: **positive** Effect: - positive effect in all tester strains: - max. mutation frequency: TA 98 = 10.3 TA 100 = 4.6 TA 104 = 2.9 Cytotoxicity: - TA 98 (5.0 µg/plate) - TA 100 (≥ 2.0 µg/plate) Controls:	DeMarini et al. 2011

Test	Bacterial strain (S. typhi-murium tester strains)	Concentration	Metabol. activa-tion	Result and Remarks	Reference
				- relevant negative and positive control Remarks: - plate incorporation test - Aroclor-induced rat liver S9 mix Deviations: Due to the positive result following deviations from the OECD TG 471 are not of decisive relevance: - no test without metabolic activation (it is known that a metabolic activation is necessary for the biological effect of DB[a,l]P) - for evaluation two instead of three plates per concentration were used at each dose level - only three bacterial strains were tested	

Table 12: DNA repair in bacteria

Test	Bacterial strain	Concentration	Metabol. activa-tion	Result and Remarks	Reference
DNA repair test (SOS chromo-test) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	E. coli PQ37	0.156 – 10'000 µg/assay	+/-	Result: **positive** Supporting study Effect: - positive only with metabolic activation from 0.156 µg/assay upwards Cytotoxicity: no	Mersch-Sundermann et al. 1992

Table 13: Mutagenicity tests in mammalian cells

Test	Cell type	Concentration	Metabol. Activa-tion	Result and Remarks	Reference
Gene mutation test (TK locus) Not in accordance with OECD TG 476 Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	MCL-5 cells - cell line derived from human B-lymphoblastoid cells - cells contain activity for five cytochromes P450 and microsomal epoxide hydrolase	0.1 - 50 ng/mL	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - positive at all tested concentrations in a dose-dependent manner Cytotoxicity: - lowest survival rate of approximately 60 % at the highest tested concentration Remarks: - no differentiation of colony size for determi-nation of small and large colonies suggestive for chromosomal aberration respectively gene	Busby et al. 1995

Test	Cell type	Concentration	Metabol. Activation	Result and Remarks	Reference
				mutation - as negative control only the historical negative control was taken into account - lack of detailed experimental data - only graphical presentation	
Gene mutation test (TK locus) Compliant to OECD TG 476 GLP: no information **Test substance: DB[a,l]P** Purity: 94 %	h1A1v2 cells - cell line derived from human B-lymphoblastoid cells - cells have been engineered to express cytochrome P450A1 (CYP1A1), an enzyme capable of metabolizing PAH	0.1 – 10 ng/mL	+ (see column 'Cell type')	**Key study** (reliable study; OECD TG 476 with deviation (see below)) <u>Result:</u> **positive** <u>Effect:</u> - positive from 0.5 ng/mL in a dose-dependent manner up to the highest tested concentration of 10 ng/mL (max. mutation frequency 8.7) <u>Cytotoxicity:</u> - from 1.0 ng/mL upwards clearly cytotoxic at the highest tested concentration with a survival rate of 19 % <u>Controls:</u> - relevant negative and positive control <u>Deviation:</u> - no differentiation of colony size for determination of small and large colonies suggestive for chromosomal aberration respectively gene mutation	Durant et al. 1996
Gene mutation test (HPRT) Not in accordance with OECD TG 476 Crucial deficiency: no positive control	V79 cells (target cells) MCF-7 cells (used as activator cells; human mammary carcinoma cell line; cytochrome P450 expressing cells)	0.005 - 0.025 µM	+ (see column 'Cell type')	<u>Result:</u> **positive** Supporting study <u>Effect</u> - dose-dependent effect from 0.005 µM upwards	Ralston et al. 1997

Test	Cell type	Concentration	Metabol. Activation	Result and Remarks	Reference
GLP: no information **Test substance: DB[a,l]P** Purity: no information				Cytotoxicity: - viability of V79 cells decreases as the dose of DB[a,l]P increased Remarks: - mammalian cell-mediated assay - lack of detailed information	
Gene mutation test (HPRT) Not in accordance with OECD TG 476 Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	V79MZh1A1 cellsV79MZh1B1 cells (hamster lung fibroblasts transfected with human cytochrome P450-1A1 (hCYP1A1) or P450-1B1 (hCYP1B1))	0.5, 1.0, 2.0 and 3.0 nM	+ (see column 'Cell type')	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - dose-dependent effect in both cell lines - cells expressing hCYP1B1 were more sensitive to mutagenesis Cytotoxicity: - cell line-dependent cytotoxicity: V79MZh1A1 cell line > V79MZh1B1 cell line Remarks: - only graphical presentation - lack of detailed experimental data	Kushman et al. 2007
Micronucleus test Not in accordance with OECD TG 487 Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P**	V79MZh1A1 cellsV79MZh1B1 cells (hamster lung fibroblasts transfected with human cytochrome P450-1A1 (hCYP1A1) or P450-1B1 (hCYP1B1))	1.0 – 1'000 nM	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - positive effect in both cell lines Cytotoxicity: - cell line dependent cytotoxicity; CYP1A1 (EC_{50}= 12 nM) > CYP1B1 (EC_{50}= 45 nM) Remarks:	Schober et al. 2006

Test	Cell type	Concentration	Metabol. Activa-tion	Result and Remarks	Reference
Purity: 99.8 %				- only graphical presentation - lack of detailed experimental data	

Table 14: Indicator effects in mammalian cell cultures

Test	Cell type	Concentration	Metabol. activa-tion	Result and Remarks	Reference
Comet assay No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: B[a,l]P** Purity: no information	MRC-5 cells (human lung fibroblast cell line) - cells capable of activating PAH to reactive metabolites	1.65, 8.27 and 16.53 µM	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - positive effect at all concentrations - the strongest effect was observed at 8.27 µM Cytotoxicity: - no cytotoxicity - information that the highest dose was established according to the highest tolerable dose criteria in a pilot experiment	Mouron et al. 2006
SCE test (sister-chromatid ex-change) Not in accordance with OECD TG 479 Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	MRC-5 cells (human lung fibroblast cell line) - cells capable of activating PAH to reactive metabolites	1.65, 8.27 and 16.53 µM	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - positive effect at all concentrations in a dose-dependent manner Cytotoxicity: - no cytotoxicity - information that the highest dose was established according to the highest tolerable dose criteria in a pilot experiment	Mouron et al. 2006
Determination of	MFC-7 cells (human	8.0 µM	+	Result: **not reliable**	Ralston et al. 1994

Test	Cell type	Concentration	Metabol. activation	Result and Remarks	Reference
DNA adducts (^{35}S-postlabelling analysis) No guideline available Crucial deficiencies: neither negative control nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	mammary carcinoma cell line) - cytochrome P450 expressing cells		(see column 'Cell type')	Study is not considered because both the negative control and the positive control are missing. <u>Effect:</u> - three major adducts and three further adducts were identified <u>Cytotoxicity:</u> - no information	
Determination of DNA adducts (^{32}P-postlabelling analysis) Crucial deficiencies: neither negative control nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	MFC-7 cells (human mammary carcinoma cell line) - cytochrome P450 expressing cells	8.0 and 16 µM	+ (see column 'Cell type')	<u>Result:</u> **not reliable** Study is not considered because both the negative control and the positive control are missing. <u>Effect:</u> - three major adducts and three further adducts spots were identified <u>Cytotoxicity:</u> - no information	Ralston et al. 1995
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control	Human mammary epithelial cells (isolated from healthy women undergoing reduction mammoplasty)	500 µM	+ (see column 'Cell type')	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - five adduct spots were identified - reference of the authors that the patterns and number of adducts resembled those detected in	Carmichael et al. 1996

Test	Cell type	Concentration	Metabol. activation	Result and Remarks	Reference
GLP: no information **Test substance: DB[a,l]P** Purity: no information				mouse skin DNA from topically treated animals (see table 17: Hughes and Phillips 1990) <u>Cytotoxicity:</u> - no information <u>Remark:</u> - only limited information is available	
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: 99 %	Mouse embryo fibroblast cell line C3H10T1/2 - cells are capable of activating PAH to reactive metabolites	0.1 µg/mL	+ (see column 'Cell type')	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - seven distinct adduct spots were identified - type of adducts: anti-DB[a,l]PDE-deoxyadenosine adduct[1] anti-DB[a,l]PDE-deoxyguanosine adduct[1] syn-DB[a,l]PDE-deoxyguanosine adduct[1] syn-DB[a,l]PDE-deoxyguanosine adduct[1] <u>Cytotoxicity:</u> - approximately 80 % cell survival	Nesnow et al. 1997
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance:**	V79MZ-h1A1 cells V79MZ-h1B1 cells (hamster lung fibroblasts) - transfected with human cytochrome P450-1A1 (hCYP1A1) or P450-1B1 (hCYP1B1)	0.1 and 1.0 µM	+ (see column 'Cell type')	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - several DNA adducts were identified in both cell lines (DB[a,l]PDE-DNA adducts[1]) - in both cell lines the number of adducts and their quantity was dose-dependently increased <u>Cytotoxicity:</u> - no information	Luch et al. 1998

Test	Cell type	Concentration	Metabol. activa-tion	Result and Remarks	Reference
DB[a,l]P Purity: no information					
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control **Test substance: DB[a,l]P** Purity: no information	HEL cells (human embryonic diploid lung fibroblast cell line) - cells capable of activating parent PAH to reactive metabolites	0.1 µM	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - three adducts were identified Cytotoxicity: - no information	Binkova et al. 2000
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	MFC-7 cells (human mammary Carcinoma cell line) - cytochrome P450 express-ing cells	1.0 and 2.0 µM	+ (see column 'Cell type')	Result: **positive** Supporting study Effect: - DNA adduct formation at both tested concen-trations Cytotoxicity: - no cytotoxicity Remark: - comparative quantitative evaluation of adduct formation	Melendez-Colon et al. 2000

[1] …-DB[a,l]P-11,12-diol-13,14-epoxide…

Table 15: Effects in vitro at isolated calf thymus DNA

Test	DNA	Concentration	Metabol. activation	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Calf thymus DNA	80 µM	+ - 3-methyl-cholanthrene-induced rat liver microsomes - Horseradish peroxidase	Result: **positive** Effect: - six adducts were identified after activation by induced rat liver microsomes - three adducts were identified after activation by horseradish peroxidase	Li et al. 1995
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Calf thymus DNA	50 µM	+ Rat liver microsomes induced by: - Aroclor 1254 - ß-naphthoflavone (microsome mediated assay)	Result: **positive** Effect: - one major adduct and seven further DNA adducts were identified for each experimental approach	Arif and Gupta 1997

Test	DNA	Concentration	Metabol. activation	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	Calf thymus DNA	0.1 µM	+ 3-methyl-cholan-threne-induced rat liver microsomes (microsome mediated assay)	Result: **positive** Effect: - nine adduct spots were identified	Jankowiak et al. 1998
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative control nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	Calf thymus DNA	10 µM	+ Aroclor 1254- in-duced rat liver micro-somes (microsome mediated assay)	Result: **positive** Effect: - one major adduct and six further DNA adducts were identified	Smith et al. 1998

Test	DNA	Concentration	Metabol. activation	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	Calf thymus DNA	80 µM	+ 3-methyl-cholanthrene-induced rat liver microsomes (microsome mediated assay)	Result: **positive** Effect: - five different spots were identified	Devanesan et al. 1999
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: 99 %	Calf thymus DNA	50 µM	+ Human cytochromes P450: 1A1 1A2 1B1 2B6 2C9 2E1 3A4 (cytochrome mediated assay with human epoxide hydrolase)	Result: **positive** Effect: - only cytochromes P450 1A1 and P450 1B1 catalysed adduct formation - P450 1A1: five major and nine minor adducts - P450 1B1: two major and four minor adducts	King et al. 1999

Test	DNA	Concentration	Metabol. activation	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiencies: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Calf thymus DNA	80 µM	+ 3-methyl-cholan-threne-induced rat liver microsomes (microsome mediated assay)	Result: **positive** Effect: - six DNA adducts were identified	Todorovic et al. 2005

4.9.1.2 In vivo data

Table 16: Mutagenicity tests in soma cells

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Gene mutation assay Not in accordance with OECD TG 488 Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P**	B6C3F1 Big Blue® mice (6 males/group)	Lung cells	Exposure: (1) single i.p. injection (2) repeated i.p. injection Doses: (1) 6 mg/kg bw (2) injection of 1.2 mg/kg bw daily for 5 d Harvest times: (1) + (2) 31 d after the	Result: **positive** Supporting study Effect: - significantly increased mutation frequency after both treatment regimens (2,4-fold increase after single injection; 2.8-fold increase after repeated injections) - difference in mutation frequency between the treatment regimens was not statistically significant	Leavitt et al. 2008

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Purity: no information			final injection	Toxicity: - no overt toxicity - all animals survived - no significant changes in weight compared to the control animals	
Gene mutation assay Not in accordance with OECD TG 488 Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	B6C3F1 Big Blue™ mice (6 males/group)	Cells of the oral cavity: - Tongue cells - Upper oral mucosa cells	Exposure: oral (topical application in the oral cavity) Doses: 3.0, 6.0 and 12 nM three times a week for 38 weeks Harvest time: 38 weeks after first admin-istration	Result: **positive** Supporting study Effect: - gene mutations were induced in tongue cells as well as in upper oral mucosa cells - dose-dependent effect in both cell types - increase in mutations relative to vehicle control reached statistical significance at the highest tested dose of 12 nmol (approximate doubling of the mutant fraction in both cell types) Toxicity: - no change in weight and physical behaviour - no mortality - no maximum tolerated dose was tested (pilot study for determination whether mutations could be induced in the oral cavity)	Guttenplan et al. 2012

Table 17: Indicator tests in soma cells

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control	Park mice (4 males/group)	- Skin cells (cells of treated skin area) - Lung cells (after treatment of skin)	Exposure: single topical treatment Dose: application of 1.0 µM/mouse (treated skin area) Harvest times: 6 h as well as 1, 2, 4, 7, 21	Result: **positive** Supporting study Effect: - induction of DNA adducts in treated skin cells as well as in lung cells - DNA from treated mouse skin yielded six	Hughes and Philipps 1990

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %			and 84 d after treatment (treated areas of skin and lungs cells were removed)	adducts - the pattern of DNA adducts in lung cells was qualitatively similar to that seen in treated skin cells but quantitative differences were evident - the majority of adducts were removed within 21 days post-treatment; low levels were found in both tissues to persist for at least three months <u>Toxicity:</u> - no information	
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Sprague-Dawley rats (4 females)	- Mammary epithelial cells - Lung cells - Liver cells - Heart cells - Pancreas cells - Bladder cells	<u>Exposure:</u> single intra-mammillary injection (under the nipple region of third, fourth and fifth mammary gland on both sides at each tested animal) <u>Dose:</u> 0.25 µM/gland <u>Harvest time:</u> 2 d after injection	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - at mammary epithelial DNA two major and five minor adducts were identified (interaction of both anti- and syn-DBPDEs[1] with adenine and guanine in DNA) - DNA of lung cells, heart cells, bladder cells and pancreas cells had a qualitatively identical adduct pattern - in liver cells four additional adduct spots were detected <u>Toxicity:</u> - no information	Arif et al. 1997a
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information	A/J mice (5 males/group)	Lung cells	<u>Exposure:</u> single i.p. injection <u>Doses:</u> 0.3, 1.5, 3.0 and 6.0 mg/kg bw <u>Harvest times:</u> between 1 and 28 d	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - six major and four minor adducts were identified (interaction of both anti- and syn-DBPDEs[1] with adenine and guanine in DNA) - maximal adduct levels occurred between 5 and 10 days after injection followed by a gradual	Prahalad et al. 1997

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Test substance: DB[a,l]P Purity: no information				decrease Toxicity: - no information	
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	A/J mice (males; number of tested animals is unknown)	Lung cells	Exposure: single i.p. injection Dose: 6.0 mg/kg bw Harvest time: 3 d after injection	Result: **positive** Supporting study Effect: - three major and seven minor adducts were identified (syn- and anti-fjord-region diolepopxide adducts of dAdo and dGuo) Toxicity: - no information	Nesnow et al. 1998
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	Parkes mice (4 males)	Skin cells	Exposure: single topical treatment (shaved areas of backs) Dose: 1.0 µM Harvest time: 24 d after treatment	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - seven adduct spots were identified Toxicity: - no information	Jankowiak et al. 1998
Determination of DNA	Sprague-Dawley	(1)	Exposure:	Result: **positive**	Arif et al. 1999

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	rats (females; number of tested animals is unknown)	- Mammary epithelial cells- Liver cells - Lung cells- Heart cells - Pancreas cells - Bladder cells (2) + (3) - Mammary Epithelial cells - Lung cells	(1) single intra-mammillary (i.m.) injection (under the nipple region of third, fourth and fifth mammary gland on both sides at each tested animal) (2) single gavage (3) single i.p. injection <u>Doses:</u> (1) 0.25 µM/gland (2) + (3) 1.5 µM <u>Harvest times:</u> (1 – 3) 6 h as well as 2, 5 and 14 d post-treatment	Supporting study <u>Effect:</u> (1) at mammary epithelial DNA one predominant and six further adducts were identified (1) DNA of lung cells, heart cells, pancreas cells and bladder cells had a qualitatively identical adduct pattern (1) in liver cells four additional adduct spots were detected (2) + (3) administration by gavage or i.p. injection resulted in essentially the same adduct patterns in mammary and lung cells as formed by the intra-mammillary route (1) – (3) quantitatively, the i.m. route resulted in significant higher adduct formation > i.p. route > gavage - adducts were both deoxyadenosine- and deoxyguanosine-derived, formed by interaction with both syn- and anti-DBPDEs[1] <u>Toxicity:</u> - no information	
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information	CENCAR mice (5 females/ group)	Skin cells (shaved area of dorsal skin)	<u>Exposure:</u> single topical treatment <u>Doses:</u> 50, 200 and 400 nM <u>Harvest times:</u> 4 and 24 h after treatment	<u>Result:</u> **positive** Supporting study <u>Effect:</u> - several DNA adducts were identified (mainly (-)-antiDB[a,l]PDE-DNA adducts[1]; small proportion of (+)-synDB[a,l]PDE-DNA adducts[1]) - DNA adducts were formed in a time- and	Melendez-Colon et al. 1999

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Test substance: DB[a,l]P Purity: no information				dose-dependent manner Toxicity: - no information	
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive controls GLP: no information **Test substance: DB[a,l]P** Purity: no information	SENCAR mice (3 females)	Skin cells (shaved area of dorsal skin)	Exposure: single topical treatment Dose: 2.0 nM (0.6 µg) harvest times: 12 and 24 h after treatment	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - four major adducts and one minor adduct were identified after 12 h and 24 h Toxicity: - no information	Marston et al. 2001
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	Swiss mice (females; number of tested animals is unknown)	Skin cells (shaved area of dorsal skin)	Exposure: single topical treatment Dose: 200 nM Harvest time: 4 h after treatment	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - several DNA adducts were identified Toxicity: - no information	Cavalieri et al. 2005
Determination of DNA	Sprague-Dawley	Mammary tissue	Exposure:	Result: **not reliable**	Cavalieri et al.

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	rats (females; number of tested animals is unknown)	cells (cells of the mammary gland areas)	single intra- mammillary injection (under the nipple region of fourth and fifth mammary gland on both sides at each tested animal) Dose: 200 nM/gland Harvest time: 24 h post-treatment	Study is not considered because both the negative control and the positive control are missing. Effect: - several DNA adducts were identified Toxicity: - no information	2005
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: no positive control GLP: no information **Test substance: DB[a,l]P** Purity: no information	C57BL/6 mice (females; number of tested animals is unknown)	Lung cells	Exposure: gavage Doses: 1.0, 5.0 and 20 mg/kg bw daily for 10 d Harvest time: at day 11 after the beginning of the gavage	Result: **positive** Supporting study Effect: - DNA adduct profiles contained four main adduct peaks (all adducts originated from intermediate formation of DBPDE[1]) - dose-dependent quantity of adduct formation Toxicity: - decrease in body and organ weights	Mahadevan et al. 2005

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Swiss mice (females; number of tested animals is unknown)	Skin cells (shaved area of dorsal skin)	Exposure: single topical treatment Dose: 200 nM Harvest time: 4 h after treatment	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - several DNA adducts were identified Toxicity: - no information	Todorovic et al. 2005
Determination of DNA adducts (^{32}P-postlabelling analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: > 99 %	Sprague-Dawley rats (females; number of tested animals is unknown)	Mammary gland cells	Exposure: single intra- mammillary injection (under the nipple region of fourth and fifth mammary gland on both sides at each tested animal) Dose: 200 nM/gland Harvest time: 24 h post-treatment	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - several DNA adducts were identified Toxicity: - no information	Todorovic et al. 2005
Determination of DNA adducts (HPLC-MS/ MS analysis)	B6C3F1 mice (females: 3/group (1), 6/group (2))	Oral tissues (collection of soft tissues of the oral cavity including	Exposure: topical into the oral cavity Doses: (1) 240 nM/d for 2 d	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing.	Zhang et al. 2011

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: not given		the buccal mucosa and the floor of the mouth as well as soft tissues attached to the hard palate; tissues were pooled for DNA adduct analysis)	(2) 24 nM, three times a week for 5 weeks Harvest times: (1) 24 h after second application (2) 48 h, 1, 2 and 4 weeks after the last application	Effect: (1) two DNA adducts were identified (2) a time-course study revealed a time-dependent disappearance pattern of both adducts after 48 h Toxicity: (1) + (2): - no information	
Determination of DNA adducts (LC-MS/MS analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information **Test substance: DB[a,l]P** Purity: ≥ 99 %	B6C3F1 mice (6 females/ group)	Ovarian cells	Exposure: topical into the oral cavity Dose: -24 nM, three times a week for 5 weeks Harvest times: - 48 h, 1, 2 and 4 weeks after the last application	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - two DNA adducts were identified Toxicity: - no information	Chen et al. 2012
Determination of DNA adducts (LC-MS/MS analysis) No guideline available Crucial deficiency: neither negative nor positive control GLP: no information	B6C3F1 mice (females: 3/group (1), 6/group (2))	Oral and tongue tissues	Exposure: oral Dose: 24 nM, three times a week for 5 weeks Harvest times: (1) 24 h after second application	Result: **not reliable** Study is not considered because both the negative control and the positive control are missing. Effect: - DNA adducts were identified in oral and tongue tissues at all harvest times Toxicity:	Zhang et al. 2014

Test	Species	Target cells	Exposure and harvest time	Result and Remarks	Reference
Test substance: DB[a,l]P Purity: no information			(2) 48 h, 1, 2 and 4 weeks after the last application	- no information	

[1] …-DB[a,l]P-11,12-diol-13,14-epoxides…

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Only those in vitro studies (see tables 11-15) / in vivo studies (see tables 16-17) are considered for the discussion of the mutagenicity of DB[a,l]P, which were characterized as 'key study' or 'supporting study'.

Exclusively positive in vitro/in vivo genotoxicity tests are available. A evaluation of these tests taking into account the quality of the test performances leads to the conclusion that only two positive in vitro mutagenicity tests (key studies) are to be considered for justification of classification of DB[a,l]P as mutagen: bacterial gene mutation test (see table 11: DeMarini et al. 2011); in vitro gene mutation test (see table 13: Durant et al. 1996)).

Although the other positive results from in vitro/in vivo testing seem to be conclusive all these tests have a crucial methodological shortcoming due to the lack of a positive control (supporting studies; see tables 11-14 and 16-17).

Experimental data *in vitro*

DB[a,l]P induces mutagenic effects in bacteria as well as in exposed proliferating cells of mammalian and human cell lines. Positive effects were also proved with indicator tests in different cell cultures as well as on isolated calf thymus DNA. Requirement for the biological activity of DB[a,l]P is its metabolic activation.

DB[a,l]P induces gene mutation in different S. typhimurium tester strains (see table 11: Devanesan et al. 1990; Busby et al. 1995; DeMarini et al. 2011) as well as DNA damage in E. coli tester strain PQ37 (see table 12: Mersch-Sundermann et al. 1992).

In mammalian cell gene mutation tests DB[a,l]P induces positive effects (see table 13) at TK locus in MC-5 cells (Busby et al. 1995) and in h1A1v2 cells (Durant et al. 1996) as well as at HPRT locus in V79 cells with MCF-7 cells as activator cells (Ralston et al. 1997).

An in vitro micronucleus test was positive (see table 13) in V79MZh1A1 cells as well as in V79MZh1B1 cells (Schober et al. 2006).

Although none of the studies include a positive control the following indicator tests showed consistent positive results in different cell cultures (see table 14): Comet assay (Mouron et al. 2006), SCE test (Mouron et al. 2006), DNA adduct formation (Carmichael et al. 1996; Nesnow et al. 1997; Luch et al. 1998; Binkova et al. 2000; Melendez-Colon et al. 2000).

The formation of DNA adducts has been demonstrated also in cell-free tests using ^{32}P-postlabelling analysis with isolated calf thymus DNA and various metabolic activation systems (see table 15: Li et al. 1995; Arif and Gupta 1997; Jankowiak et al. 1998; Smith et al. 1998; Devanesan et al. 1999; King

et al. 1999; Todorovic et al. 2005). These results are used only as additional information without relevance for a classification proposal due to methodological deficiencies (lack of negative / positive controls) and the fact that these tests were conducted outside of cell systems.

Experimental data *in vivo*

DB[a,l]P induces gene mutations as well as DNA adducts in soma cells.

Gene mutations (see table 16) were induced in lung cells (Leavitt et al. 2008) as well as in cells of the oral cavity (tongue cells and upper oral mucosa cells) (Guttenplan et al. 2012).

Using the ^{32}P-postlabelling analysis DNA adducts (see table 17) were detected in mammary epithelial cells (Arif et al. 1997a; Arif et al. 1999) as well as in cells of skin (Hughes and Philipps 1990; Melendez-Colon et al. 1999), lung (Hughes and Philipps 1990; Arif et al. 1997a; Prahalad et al. 1997; Nesnow et al. 1998; Arif et al. 1999; Mahadevan et al. 2005), liver (Arif et al. 1997a; Arif et al. 1999), heart (Arif et al. 1997a; Arif et al. 1999), pancreas (Arif et al. 1997a; Arif et al. 1999) and bladder (Arif et al. 1997a; Arif et al. 1999).

Validity of the test systems

The available in vitro and in vivo tests are qualitatively different in their test performances.

A evaluation of the mutagenicity/genotoxicity tests with DB[a,l]P taking into account the quality of the test performances leads to the conclusion that only the positive results of two in vitro mutagenicity tests can be considered reliable: A gene mutation test in h1A1v2 cells (Durant et al. 1996) was carried out in accordance with the corresponding OECD TG 476. Although a bacterial gene mutation test (DeMarini et al. 2011) was not carried out in full compliance with the corresponding OECD TG 471 the deviations (two instead three plates per were used for each concentration; only three instead of five bacterial were tested) do not impact the reliability of the positive data obtained from these study.

For all other exclusively positive genotoxicity tests the lack of positive controls is a crucial methodological shortcoming. The results of these tests are considered as not fully reliable. Relevant controls (positive as well as negative controls) have to be included in toxicological tests in according to EU/OECD guidelines. Also for internationally accepted testing procedures for which no EU/OECD guideline exist the use of controls is a standard for the detection of the functionality and reliability of tests.

According to CLP Regulation and the ECHA guidance to CLP only fully reliable positive results of well conducted and scientifically validated tests are relevant for the justification of toxicological classification of a substance. According to these criteria only two positive in vitro tests (Durant et al. 1996; DeMarini et al. 2011) can be considered for the justification of classification of DB[a,l]P as mutagen.

Read-across approach

A read-across approach is justified between DB[a,l]P and B[a]P as well as CHR due to their similar chemical structure activity relationship which leads to induction of mutagenic effects.

B[a]P and a number of further PAH, e.g. DB[a,l]P, DB[a,h]P, DB[a,i]P and CHR have shown mutagenic/genotoxic effects in standard assays in vitro and in vivo (IPCS 1998; SCF 2002; FAO/WHO

2006; EFSA 2008; IARC 2010; Benford et al. 2010). However so far, only B[a]P and CHR are classified as mutagenic. B[a]P (CAS 50-32-8) is classified/labelled as Cat. 1B, H340 (germ cell mutagen), whereas CHR (CAS 218-01-9) is classified/labelled as Cat. 2, H341 (suspected germ cell mutagen) according to CLP Regulation.

In accordance with the CLP Regulation (Annex I, point 3.5.2.2, p. 149) it has been checked whether a read-across approach can be used for a classification proposal of DB[a,l]P referring to the structurally similar substances B[a]P and CHR that are classified as germ cell mutagens: 'Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.'

DB[a,l]P, B[a]P and CHR consists of four (CHR), five (B[a]P) or six (DB[a,l]P) fused benzene rings. Depending of the number of benzene rings and the resulting steric effects the substances can be distinguished regarding their metabolic reactive centres bay region (B[a]P and CHR) or fjord region (DB[a,l]P). When the substances are metabolized reactive electrophilic bay or fjord dihydrodiol epoxide enantiomers are generated that are able to bind covalently with guanine nucleotides (bay region epoxides) and adenine nucleotides (fjord region epoxides) in cellular DNA to form pre-mutagenic covalent adducts in mammalian cells and tissues that can, if not repaired, ultimately contribute to mutagenic effects (Lin et al. 2001; Munoz and Albores 2011; Tang et al. 2012; Rodriguez et al. 2014).

The diol epoxide pathway is the most widely accepted pathway of PAH activation to yield DNA adducts (Xue and Warshawsky 2005; Chen et al. 2012). The metabolic activation of PAH occurs in the same way to produce a diol epoxide irrespective of the molecular structure and the different steric conditions at the reactive centres. The pathway is catalysed mainly by several enzymes such as CYPs and epoxide hydrolases. Metabolic activation finally leads to the formation of electrophilic diolepoxides, which belong to the most potent mutagens reported so far **(**Xue and Warshawsky 2005).

There is extensive information on the metabolic activation of PAH as well as their binding to DNA. Especially often B[a]P and DB [a,l]P were used as so called 'model PAH' for such examinations. For example, DNA adduct studies (Doehmer et al. 1995; Luch et al. 1998) have shown that DB[a,l]P and B[a]P are metabolized and activated at their reactive centres primarily in the 11,12,13,14 or 7,8,9,10 positions of the aromatic ring system to dihydrodiol-epoxides e.g. in vitro in V79 cell lines expressing CYP 1A1 (V79-hCYP1A1) and CYP 1B1 (V79-hCYP1B1 cells). Other metabolism and DNA-binding studies performed with different cell lines or liver preparations from rats pretreated with different inducers of cytochrome P450 (CYP) enzymes such as 3-methylcholanthrene (MC) or Aroclor 1254 revealed that DB[a,l]P is predominantly metabolized to genotoxic fjord region syn- and/or anti-11,12-dihydrodiol-13,14-epoxides (syn- and anti-DB[a,l]PDE). This activation resembles the stereoselectivity found for other PAH such as B[a]P (Shou et al. 1996; Luch et al. 1997; King et al. 1999; Schober et al. 2006). Information on diol epoxides of CHR as well as their metabolism to DNA binding species is given for example by Hall et al. (1988), Glatt et al. (1993) as well as Patel and Arif (2014).

Different steric arrangements in the respective PAH ring system influence the binding affinity of PAH to DNA. The bay region containing PAH (e.g., B[a]P and CHR) are characterized by a planar aromatic ring system whereas the fjord region containing PAH (e.g., DB[a,l]P) show non-planarity in the aromatic ring system (Katz et al. 1998). The resulting topology of DNA adducts influence the distortions and stabilities of double-stranded DNA, and hence their processing by repair mechanisms (Luch 2009; Rodriguez et al. 2014). Accordingly, the adduct repair is conformation-dependent. The extent of the activity of nucleotide excision repair enzymes is influenced by the steric features at the reactive centres (Dreij et al. 2005, Zhong et al. 2010).

DNA adducts derived from the PAH containing bay regions are repaired more rapidly and effectively than adducts derived from PAH containing fjord regions. It was shown that DB[a,l]P exerts much more genotoxic effects than B[a]P in vitro (Hughes and Phillips 1990; Melendez-Colon et al. 2000), respectively in an animal model system (Melendez-Colon et al. 2000).

Summary:
Due to their comparable chemical structures and the resulting metabolic activity a read-across approach between DB[a,l]P and B[a]P as well as CHR is sufficiently justified. The read-across approach is based on following common substance properties:
- The substances belong to the chemical group of PAH.

- The substances require metabolic activation for the induction of mutagenic/genotoxic effects.

- Reactive centres of the substances are the so called fjord region (DB[a,l]P) or bay region (B[a]P and CHR).

- Reactive electrophilic bay or fjord dihydrodiol epoxide enantiomers are generated as metabolic products.

- The dihydrodiol epoxides bind covalently to specific targets in cellular DNA to form adducts with DNA nucleotides.

- Unrepaired DNA adducts can led to mutagenic effects as a result of cell division.

- PAH induce mutagenic/genotoxic effects in vitro and in vivo after metabolic activation regardless of whether the reactive centre is a bay region or a fjord region.

Additional information on classification of the structurally similar substances DB[a,i]P and DB[a,h]P

For the classification proposal for DB[a,i]P and DB[a,h)]P as Muta.2 agreed by RAC at its 41th meeting (June 2017) toxicological information are available which are comparable to those for DB [a, l] P. Guideline-compliant positive in vitro gene mutation tests (HPRT and TK-locus) characterise the substances as in vitro mutagens. Their chemical structure activity relationship to known mutagenic PAHs (B[a]P (Muta. 1B) and chrysene (Muta. 2)) were used as supporting criterion for justification of classification of DB[a,i]P and DB[a,h)]P as Category 2 mutagens in accordance with the CLP Regulation (Annex I, point. 3.5.2.2, p 149). A variety of not guideline-compliant positive in vitro/in vivo genotoxicity support classification proposal.

4.9.5 Comparison with criteria

Category 1 mutagen

According to the CLP Regulation (see 3.5.2.2, p. 145) substances that are known to induce heritable mutations or are to be regarded as if they induce heritable mutations in germ cells of humans are classified as Category 1 mutagen:

Category 1A: There is positive evidence from human epidemiological studies.

Category 1B: There are positive results from
- *in vivo* heritable germ cell mutagenicity tests in mammals or
- *in vivo* soma cell mutagenicity tests in mammals (in combination with evidence that the substance has potential to cause mutations to germ cells) or

- tests showing mutagenic effects in germ cells of humans without demonstration of transmission to progeny.

No data are available which justify a classification of DB[a,l]P as mutagen Category 1 in accordance with the CLP Regulation (Annex I, point 3.5.2.2, p. 145).

Category 2 mutagen

According to the CLP Regulation (Annex I, point 3.5.2.2, p. 145) the classification of a substance as mutagenic Category 2 is based on: '… positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments' obtained from:
- somatic cell mutagenicity tests in vivo, in mammals or
- other in vivo somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

The ECHA guidance to CLP Regulation additionally explains (point 3.5.2.4, p. 287) that:
- 'Classification in Category 2 may be based on positive results of a least one in vivo valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in soma cells.'
- 'A Category 2 mutagen classification may also be based on positive results of a least one in vivo valid mammalian genotoxicity test, supported by positive in vitro mutagenicity results.'

Taking into account these criteria neither a valid in vivo mammalian somatic cell mutagenicity test nor a valid in vivo mammalian genotoxicity test is available for DB[a,l]P. The available guideline-compliant in vitro gene mutation tests alone are not sufficient as justification for classification of DB[a,l]P as a Category 2 mutagen.

The CLP Regulation (Annex I, point 3.5.2.2, p. 149) also notes: 'Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.' These criteria are fulfilled for DB[a,l]P. The substance induces gene mutations in vitro and is structurally similar to the germ cell mutagen B[a]P.
The read-across approach clearly supports the relevance of the consistently observed positive mutagenic / genotoxic effects in vitro and in vivo in soma cells induced by DB[a,l]P despite the lack of positive controls in most of the positive mutagenicity tests. It is reasonable to conclude that the classification of B[a]P and CHR as germ cell mutagen can be used as a supporting criterion for justification of classification of DB[a,l]P as a mutagen.

4.9.6 Conclusions on classification and labelling

The currently available positive results of the guideline-compliant in vitro gene mutation tests of DB[a,l]P, combined with the read-across approach to the germ cell mutagens B[a]P and CHR, are sufficient for classification of DB[a,l]P as Category 2 mutagen, H341 in accordance with CLP Regulation.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Dibenzo[def,p]chrysene induced mutagenic effects in bacteria and in exposed proliferating cells of mammalian and human cell lines. Positive effects were also observed using indicator tests in different cell cultures as well as on isolated calf thymus DNA. These effects were only observed in the presence of an exogenous metabolic system.

Clastogenic effects (induction of micronuclei) were induced by dibenzo[def,p]chrysene as well as genotoxic effects (DNA adducts, SCE) in somatic tissues in both conventional and research studies.

In vivo studies carried out in mice showed the induction of gene mutations in lung cells and also cells of the oral cavity (tongue cells and upper oral mucosa cells).

Amongst all the studies, only two were carried out in accordance with EU/OECD test guidelines; one was a bacterial gene mutation test in *S. typhimurium* strains TA 98, TA100 and TA104 and the other was a mammalian cell gene mutation test assayed at the TK locus. Both of these tests yielded positive results.

In the opinion of the DS, taking into account the CLP Regulation and associated guidance, the quality and reliability of most of the available studies was below the required standard. The DS considered that the lack of appropriate controls in all the other tests was a crucial methodological shortcoming. Consequently, even though a significant number of positive *in vitro* and *in vivo* studies had been conducted with dibenzo[def,p]chrysene, the results of these studies alone were insufficient to support classification.

To supplement these studies, the DS argued that the structural similarity of dibenzo[def,p]chrysene to B[a]P and chrysene justified its classification as a mutagen. B[a]P has a harmonised classification for germ cell mutagenicity in Category 1B, chrysene in Category 2. The DS cited several reviews from international bodies that concluded B[a]P and chrysene have produced mutagenic/genotoxic effects in standard assays *in vitro* and *in vivo.*

B[a]P, chrysene and dibenzo[def,p]chrysene consist of four, five or six fused benzene rings (respectively). Depending of the number of benzene rings and the resulting steric effects the substances can be distinguished by the metabolic reactive centres they possess: "bay region" only (B[a]P and chrysene) or including a "fjord region" (dibenzo[def,p]chrysene). When the substances are metabolised, reactive electrophilic bay or fjord dihydro-diol epoxide enantiomers are generated that are able to bind covalently with guanine nucleotides (bay region epoxides) and adenine nucleotides (fjord region epoxides) in cellular DNA to form pre-mutagenic covalent adducts in mammalian cells and tissues that can, if not repaired, ultimately contribute to mutagenic effects.

The diol epoxide pathway is the most widely accepted pathway of PAH activation to yield DNA adducts. Such activation of PAHs occurs in the same way to produce a diol epoxide irrespective of the molecular structure and the different steric conditions at the reactive centres. The pathway is catalysed mainly by several enzymes such as cytochromes P450 and epoxide hydrolases. Metabolic activation leads to the formation of electrophilic diol epoxides, which are direct-acting mutagens.

Research studies have shown that different steric arrangements in the respective PAH ring system influence the binding affinity of a PAH to DNA. The bay region-containing PAHs (e.g., B[a]P and chrysene) are characterised by a planar aromatic ring system whereas the fjord region containing PAHs (e.g., dibenzo[def,p]chrysene) show non-planarity in the aromatic ring system. The resulting topology of DNA adducts apparently influences the distortions and stabilities of double-stranded DNA, and hence their processing by repair mechanisms. Accordingly, the adduct repair is conformation-dependent. The extent of the activity of nucleotide excision repair enzymes is influenced by the steric features at the reactive centres. DNA adducts derived from the PAHs that containing only bay regions are repaired more rapidly and effectively than adducts derived from PAHs with fjord regions. It has been shown that dibenzo[def,p]chrysene exerts a more potent genotoxic effect than B[a]P *in vivo* in an animal model system.

Due to their chemical structures and the resulting metabolic activities at the bay or fjord-region(s), the DS stated that a read-across approach between dibenzo[def,p]chrysene, B[a]P and chrysene was sufficiently justified. The read-across was based on the following common properties of the 3 substances:

- The substances belong to the same chemical group as other PAH.
- The substances require metabolic activation for the induction of mutagenic/genotoxic effects.
- Reactive centres of the substances are the so-called fjord region (dibenzo[def,p]chrysene) or bay region (B[a]P and CHR).
- Reactive electrophilic bay or fjord dihydrodiol epoxide enantiomers are generated as metabolic products.
- The dihydrodiol epoxides bind covalently to specific targets in cellular DNA to form adducts with DNA nucleotides.
- Unrepaired DNA adducts can lead to mutagenic effects as a result of cell division.
- PAHs are known to induce mutagenic/genotoxic effects *in vitro* and *in vivo* after metabolic activation regardless of whether the reactive centre is a bay region or a fjord region.

In the view of the DS, available positive results of two guideline-compliant *in vitro* gene mutation tests of dibenzo[def,p]chrysene, combined with the read-across approach to the germ cell mutagens B[a]P (Cat. 1B) and chrysene (Cat. 2), are sufficient for classification of DB[a,l]P as Category 2 mutagen, H341 in accordance with CLP Regulation.

Comments received during public consultation

One Member State wrote in support of this proposal.

Assessment and comparison with the classification criteria

The potential mutagenicity of dibenzo[def,p]chrysene has been studied *in vitro* and *in vivo.*

Twenty-five *in vitro* studies were evaluated and included 3 Ames tests, 1 DNA repair test in bacteria, 4 *in vitro* mammalian gene mutation assays, a micronucleus test, a comet assay, an SCE test and 14 studies to determine DNA adduct formation (in both mammalian cell cultures and calf thymus DNA). Given the limited nature of the study reports in the open literature, RAC agrees that only 2 of the 25 *in vitro* studies are guideline-compliant and can be considered as reliable when judged against current regulatory standards. These tests are an Ames test and a gene mutation test (TK locus in h1A1v2 cells).

The genotoxic potential of dibenzo[def,p]chrysene was further assessed in 2 gene mutation assays in mice and 16 *in vivo* DNA adduct formation assays. None of these studies conformed to a current regulatory standard. Additionally, the CLH report included summaries of 7 tumour initiator-promoter assays in mice, in which dibenzo[def,p]chrysene was used as the initiator. Positive initiation of tumours in these tests is indicative of mutagenic activity.

Given the structural similarity to other PAHs, notably B[a]P and chrysene, RAC agrees that it is appropriate to use information on these two PAHs to further support the hazard assessment of dibenzo[def,p]chrysene.

In vitro studies

Three mutagenicity assays with standard *S.typhimurium* tester strains were summarised in the CLH report. Only one of these was performed according to OECD test guidelines but all gave positive results with dibenzo[def,p]chrysene. Like other mutagenic PAHs, positive results occurred only in the presence of exogenous metabolic activation. Following incubation with 0.1–5.0 µg/plate dibenzo[def,p]chrysene, positive results were obtained in strains TA98, TA100 and TA104. Positive and negative controls behaved accordingly. In support of this, a positive result was also reported in a bacterial DNA repair test (an SOS Chromotest).

In vitro mammalian cell tests performed with dibenzo[def,p]chrysene included four gene mutation studies. Two of the gene mutation studies were carried out at the TK locus of either MCL-5 cells or a human B-lymphoblastoid cell line (h1A1v2), and two measured mutations at HPRT locus in V79 cells. All of these gene mutation tests gave positive results.

Only the mammalian cell gene mutation test with h1A1v2 cells was performed according to the OECD test guideline. These cells are specifically engineered to express cytochrome P450 1A1 (CYP1A1), an isoenzyme especially relevant for the metabolic activation of PAHs. The cells were exposed to dibenzo[def,p]chrysene at concentrations of 0.1 - 10 ng/mL, and mutations at the TK-locus were quantified. Mutation frequency was increased from a concentration of 0.5 ng/mL in a dose-dependent manner up to the highest concentration of 10 ng/mL. Cytotoxicity was observed from a concentration of 1.0 ng/mL upwards and survival rate at the highest tested concentration was 19 %. Positive and negative controls behaved accordingly.

A non-guideline *in vitro* micronucleus test also gave a positive result. This was conducted in a Chinese hamster V79 lung cell line in which human cytochrome P450 1A1 or 1B1 had been transfected.

The consistent nature of the results provides compelling evidence of benzo[rst]pentaphene's mutagenic potential.

A comet assay and an SCE test performed with dibenzo[def,p]chrysene, both carried out in MRC-5 cells are available as well as 14 studies to determine DNA adducts; 7 of these in mammalian cell lines and 7 utilised isolated calf thymus DNA. The results of all these studies were positive, except for two DNA adduct studies in MFC-7 cells which were deemed not reliable.

In conclusion, dibenzo[def,p]chrysene has mutagenic potential in a variety of bacterial and mammalian *in vitro* test systems, in the presence of an appropriate oxidative metabolic activation system. It has similar activity to that of other mutagenic PAHs, including B[a]P and chrysene.

In vivo studies

Short summaries of 16 studies to determine the presence of DNA adducts in a variety of somatic cells in rats and mice were included in the CLH report. Animals were exposed to dibenzo[def,p]chrysene in several different ways, orally by gavage, by topical application or via intra-peritoneal (i.p.) or intra-mammary gland injection. Nine of the studies weren't deemed reliable but of the 5 that were, the results were all positive for adduct formation in the cell types tested.

Two gene mutation assays were conducted to investigate the genotoxicity of dibenzo[def,p]chrysene in B6C3F1 Big Blue® mice. Neither study was conducted in accordance with OECD test guidelines, notably because no positive control was included.

In the first study, male mice (6/group) were exposed by i.p. injection to either a single dose of 6 mg/kg bw or repeated doses of 1.2 mg/kg bw/day for 5 days dibenzo[def,p]chrysene. Lung cells were harvested 31 days after the final injection and, following the assay for mutation, a positive effect was seen following both treatment regimens. After the single dose, a 2.4-fold increase in mutation frequency was observed over that in control animals, and after repeated dosing a 2.8 fold increase in mutation frequency was seen. There was no overt toxicity or mortality produced by the test substance.

In a second study, male mice (6/group) received a topical application of 3, 6 or 12 nM dibenzo[def,p]chrysene in the oral cavity, three times/week for 38 weeks. The target cells in this study were the tongue and upper oral mucosa cells; they were harvested 38 weeks after the first administration. The results showed that gene mutations were induced in both types of cells. The effect was dose-dependent in both cell types and the increase in mutations relative to the vehicle control reached statistical significance at the highest dose tested (12 nM). At this dose the mutant fraction of cells was approximately doubled in both cell types. No changes in weight or physical behaviour of the animals were noted. There was no mortality.

Although not strictly performed to a regulatory standard protocol, these 2 studies together with the observations of DNA adducts in exposed animals, provide compelling evidence that the *in vitro* mutagenicity of dibenzo[def,p]chrysene can also occur in somatic cells *in vivo*.

The CLH report also summarised the results of 9 mouse skin tumour initiation-promotion studies in which dibenzo[def,p]chrysene was employed as the initiator. All gave positive results for tumour-initiating activity regardless of single or multiple doses (i.e. increased incidence of skin papilloma compared to controls). Activity as an initiator in these assays is strongly indicative of mutagenic activity and the positive responses with dibenzo[def,p]chrysene support the outcomes of the *in vivo* and *in vitro* genotoxicity studies.

Overall, these *in vivo* data provide strong evidence of the mutagenicity of dibenzo[def,p]chrysene.

Similarity to B[a]P and chrysene (see also RAC general comment, above)

Dibenzo[def,p]chrysene shares structural and common mechanistic properties with the PAHs B[a]P and chrysene, both of which already carry a harmonised classification for germ cell mutagenicity. Notably, all 3 substances possess highly conjugated aromatic structures and common reactive centres called bay or fjord regions, depending on the steric arrangement of the PAH ring system. They all require metabolic activation at bay regions for the induction of mutagenic/ genotoxic effects. Electrophilic dihydrodiol epoxides are formed as common breakdown products of all these PAHs following oxidative metabolism at the bay and fjord regions.

Classification of dibenzo[def,p]chrysene

There are no data on human germ cell mutagenicity with dibenzo[def,p]chrysene, therefore Category 1A is not appropriate.

The *in vitro* and *in vivo* genotoxicity data are consistently positive and reproducible across the different study types. The positive studies are further supported by 9 positive initiation-promotion assays in mice, which gave results indicative of the mutagenic activity of dibenzo[def,p]chrysene. However, in the absence of data from *in vivo* studies investigating the potential effects of dibenzo[def,p]chrysene on the DNA of germ cells, or demonstrating its ability to interact with the genetic material of germ cells, the criteria for category 1B are also not met.

In accordance with the criteria in Annex I of the CLP Regulation, a category 2 classification is appropriate. This is based on an overwhelming weight of positive *in vitro* and *in vivo* data from mutagenicity and other relevant studies with dibenzo[def,p]chrysene itself and on its structural and mechanistic similarity to the established PAH mutagens chrysene, B[a]P, dibenzo[b,def]chrysene and benzo[rst]pentaphene.

Overall, in agreement with the DS proposal, the RAC opinion is for classification of dibenzo[def,p]chrysene in **Category 2 for germ cell mutagenicity (H341: suspected of causing genetic defects)**.

4.10 Carcinogenicity

There are no key studies/standard carcinogenicity studies that were in accordance to the EU/OECD standard test guidelines designed for carcinogenicity.

The carcinogenic potential of DB[a,l]P was assessed by weight of evidence consideration of all available (non-guideline) studies on its carcinogenic effects.

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Table 18: Results from oral carcinogenicity studies

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Carcinogenicity study of repeated oral administration (gavage) no guideline Dose group: 2 Control group: 2 Administration once daily, 5x/wk for 3 wk; post exposure observation up to 12 months Mice were monitored twice weekly and killed whenever a sudden weight loss (>20 % in a week) or appearance of tumours (>1 cm) Surviving mice were killed at 12 months Complete necropsy, histology of lungs, thymus, lymph nodes, spleen, liver, pancreas, kidneys, adrenal glands, intestine, uterus and ovaries on 17 of the wild-type mice and all of the P450 1B1-null mice	**Mouse** 'wild-type' (mixed genetic background of C57Bl/6 and 129/Sv) female (7 wk old) 18/group P450 1B1-null knockout female (7 wk old) 13/group Control groups of both (2) genotypes: Each 100 µL corn oil, 27	**DB[a,l]P** was synthesized Purity: 99.8 % by HPLC Vehicle: Corn oil	Once daily 1.07 mg/kg (~32 µg/ mouse) dissolved in 100 µL corn oil, 5x/wk for 3 wk Study duration at death/ sacrifice: 12 months	**Positive** **DB[a,l]P induced benign and malignant tumours in various organs, e.g. ovaries, lymphoid tissues and skin of mice after repeated oral application of 1.07 mg/kg bw/d for up to 3 weeks** **Tumour incidence/Tumour rate:** <u>Wild-type mice:</u> Survival rate: 11/18 (61 %) 17/17 (100 %), benign and malignant tumours Ovary (benign and malignant): 12/17 (72 %) Skin (papilloma): 8/17 (47 %) Malignant lymphoma: 5/17 (29 %) Uterus (benign and malignant): 5/17 (29 %) Liver adenoma: 1/17 (6 %) <u>P450 1B1-null knockout mice:</u> Survival rate: 12/13 (92 %) 8/13 (62 %), benign tumours Uterus (endometrial cystic hyperplasia: 5/13 (38 %) Lung adenomas 5/13 (38 %); Follicular lymphoma: 1/13 (8 %); Liver haemangioma: 1/13 (8 %) Control (solvent): wild-type mice: 4 tumours/27 (15 %); in detail: 1 lung adenoma; 1 liver adenoma; 1 follicular lymphoma; 1 endometrial cystic hyperplasia P450 1B1-null knockout mice: no data	Buters et al. 2002
			Application on	**Positive** **DB[a,l]P induced oral squamous cell carcinoma in**	Schwartz et al. 2004

Carcinogenicity study by painting on the tongue no guideline Oral cancer model Dose group: 5 Control group: 2 painting on the tongue 5x/wk for 30 wk Complete necropsy, for histology oral tissues and tissues from the head and neck were fixed in 10 % buffered formalin, tissue sections were stained with H&E 3 animals after week 1, 6, 10, 25 for tongue histology	**Hamster** Golden Syrian hamsters Female (4 wk old; 70 g bw) 7/group Control: 0.25 % acetone, 4	**DB[a,l]P** Purity: pure, commercial grading Vehicle: 0.25 % acetone	the tongue of 0.01 µmol (3 µg) dissolved in 0.25 % acetone 5x/wk for 30 wk Study duration: 30 wk	**Golden Syrian hamster after repeated mucosal application on the tongue** <u>Histopathology of tongue :</u> <u>After 1 wk:</u> Small discrete areas of heterochromatic cells, hyperplasia, anaplasia, and growth into adjacent connective tissue of tongue mucosa <u>After 6 wk:</u> Moderate to severe dysplastic changes (e.g. pleomorphism, anaplasia, hyperplasia, hyperchromatism, mitotic figures, hyperkeratosis) <u>After 10 wk:</u> Severe dysplastic changes (e.g., pleomorphism, anaplasia, hyperplasia, hyperchromatism, mitotic figures, hyperkeratosis) with extensive proliferation into underlying connective tissue <u>After 25 wk:</u> **→Development of oral squamous cell carcinoma** (well differentiated squamous cell carcinoma with bizarre mitoses, hyperchromatism, pleomorphism, extending into adjacent connective tissue) <u>After 25 wk:</u> 5/7 (71 %) (total number of tumour: 15) <u>After 30 wk:</u> 6/7 (86 %) (total number of tumour: 18) **Control (vehicle):** After 25 wk: No tumour (0/4) After 30 wk: No tumour (0/4)	

4.10.1.2 Carcinogenicity: inhalation

No studies available.

4.10.1.3 Carcinogenicity: dermal

Table 19: Results from carcinogenicity studies in mice, dermal application

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Carcinogenicity studies, dermal application					
Carcinogenicity study by repeated topical application to the skin no guideline 9 synthesized and/or highly purified hexacyclic aromatic hydrocarbons were tested for carcinogenicity on mouse skin Painting on the skin 3x/wk, for 12 months + 3 months treatment-free period Animals monitored weekly by palpation for tumour development, killed when tumours (≥ 1 mm^3) and persisted for 4-5 weeks Complete necropsy, histology of skin and other selected tissues	**Mouse** Swiss albino Ha/ICR/Mil Female 20/group Control: p-dioxane, 20	**DB[a,l]P** purified by chromatography followed by recrystallization Vehicle: p-dioxane	0.05 or 0.1 % in p-dioxan 3x/wk, for 12 months study duration at death/ sacrifice: 15 months	**Positive** **DB[a,l]P induced benign and malignant skin tumours at high incidences after repeated dermal application of low doses** **Tumour development on the skin:** **0.05 % (≈ 0.86 mg/kg bw/d) DB[a,l]P)** 17/20 (**85 %**; squamous cell papilloma) 17/20 (**85 %**; squamous cell carcinoma) Mean latency period: 245 days Treatment in months: Mice with tumours (squamous cell papilloma/squamous cell carcinoma) / survivors: 5 mo: (1/0) / 18; 6 mo: (3/2) / 15 7 mo: (7/6) / 10; 8 mo: (10/10) / 7 9 mo: (13/13) / 4; 10 mo: (15/15) / 2 11 mo: (17/17) / 0 **0.1% (≈ 1.71 mg/kg bw/d) DB[a,l]P)** 18/20 (**90 %**; squamous cell papilloma) 18/20 (**90 %**; squamous cell carcinoma)	Hoffmann and Wynder 1966

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Mean latency period: 210 days Treatment in months: Mice with tumours (squamous cell papilloma/squamous cell carcinoma) / survivors: 5 mo: (1/0) / 18; 6 mo: (6/4) / 15 7 mo: (10/8) / 10; 8 mo: (13/12) / 5 9 mo: (16/15) / 2; 10 mo: (17/16) / 1 11 mo: (17/17) / 1; 12 mo: (17/17) / 1 13 mo: (18/18) / 1; 14 mo: (18/18) / 0 **Control (vehicle):** No skin tumour (0/20)	
Carcinogenicity study by repeated topical application to the skin no guideline Painting on mouse skin 3x/wk, for up to 7 months Animals monitored weekly by palpation for tumour development, killed when tumours (≥ 1 mm^3) and persisted for 4-5 weeks Complete necropsy	**Mouse** Swiss albino Ha/ICR/Mil Female 19-21/group Positive-control: 0.1 or 0.05 % B[a]P, 20	**DB[a,l]P** Pure Synthesis starting from benzanthrone and benzyl- bromide through 6-benzyl benzanthrone without using metal halides Vehicle: p-dioxane	0.001, 0.005, 0.01, 0.05 or 0.1 % in 100 µL p-dioxane 3x/wk 0.001 %: (55x) 0.005 %: (40x) 0.01 %: (24x) 0.05 %: (7x) 0.01 %: (7x) study duration at death/ sacrifice: 7 months	**Positive** **DB[a,l]P induced skin tumours at high incidences after repeated dermal application of low doses** **Tumour development on the skin:** **0.001 % (≈0.017 mg/kg bw/d)** Average latent period: 93 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors After 1 mo: 0/20; 2 mo: 1/20; 3 mo: 9/20; 4 mo: 19/20; 5 mo: 20/19; 6 mo: 20/18; 7 mo: 20/9 **0.005 % (≈0.086 mg/kg bw/d)** Average latent period: 62 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors After 1 mo: 0/19; 2 mo: 7/19; 3 mo: 19/19; 4 mo: 19/18; 5 mo: 19/15; 6 mo: 19/11; 7 mo: 19/7	Masuda and Kagawa 1972

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
No histology data				**0.01 % (≈0.17 mg/kg bw/d)** Average latent period: 66 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors After 1 mo: 0/21; 2 mo: 9/21; 3 mo: 20/21; 4 mo: 21/20; 5 mo: 21/18; 6 mo: 20/0 **0.05 % (≈0.86 mg/kg bw/d)** Average latent period: 56 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors After 1 mo: 0/20; 2 mo: 12/20; 3 mo: 17/20; 4 mo: 19/11; 5 mo: 19/7; 6 mo: 19/0 **0.1 % (≈1.71 mg/kg bw/d)** Average latent period: 77 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors After 1 mo: 0/21; 2 mo: 7/19; 3 mo: 12/17; 4 mo: 16/11; 5 mo: 16/7; 6 mo: 16/0 **Positive-control (**B[a]P**):** 0.1 % B[a]P: After 7 mo: 19/0; Average latent period: 130 days Treatment in months: Mice with skin tumours (macroscopic findings as nodules) / survivors 0.05 % B[a]P: After 7 mo: 16/1; Average latent period: 161 days	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Carcinogenicity study by single topical application to the skin no guideline Comparative study of several dibenzo[a]pyrenes Dose group: 1 Control group: none Single application (painting) on mouse skin Animals were monitored weekly by palpation for tumour development mice were killed after 27 experimental weeks complete necropsy, for histology skin tissues were fixed in 10 % buffered formalin	**Mouse** SENCAR[2] derived from SENsitivity to CARcinogenesis Female (8 wk old) 24/group No control	**DB[a,l]P** Purity: >99 % Vehicle: Acetone	100 nmol (30 µg) in 100 µL of acetone study duration at death/ sacrifice: 27 weeks	**Positive** **Significant tumour-initiating activity on mouse skin after single dose of** **100 nmol (30 µg) DB[a,l]P/mouse** (≈ 1.5 mg/kg bw) **Tumour development on the skin after 27 weeks:** 7/24 mice (29 % tumour-bearing mice) 4/7 squamous cell papilloma 3/7 squamous cell carcinoma	Cavalieri et al. 1991
	Mouse	**DB[a,l]P**	1, 4 or 8 nmol	**Positive**	Higginbotham et al. 1993

[2] The SENCAR mouse strain was selectively bred for eight generations for sensitivity to skin tumour induction by the two-stage tumourigenesis protocol using 7,12-dimethylbenz(a)anthracene (DMBA) as the initiator and 12-0-tetradecanoylphorbol-13-acetate (TPA) as the promoter. The SENCAR mouse was derived from crossing Charles River CD-1 mice with skin tumour-sensitive mice (STS) (Slaga 1986; Lynch et al. 2007).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Carcinogenicity study by repeated topical application to the skin no guideline Comparative study of several dibenzo[a]pyrenes Dose group: 3 Control group: 1 2x/wk painting on shaved dorsal mouse skin, for up to 40 weeks Monitored weekly by palpation for tumour development; mice were killed when tumours reached 2 cm in diameter Surviving mice were killed after week 48 Complete necropsy; for histology tissues from treated area, lungs, liver, kidneys, spleen, urinary bladder, uterus and ovaries, other grossly abnormal tissue were fixed in 10 % buffered formalin, sections 5µm thick, stained with H&E	Swiss mice (Eppley Colony) Female (8 wk old) 22-27/group Control: 100 µL acetone, 27	Purity: >99 % by HPLC Vehicle: Acetone	(0.3, 1.2 or 2.4 µg) in 100 µL acetone, 2x/wk, for 40 wk Study duration at death/ sacrifice: 48 wk	**DB[a,l]P induced significant skin tumours (squamous cell carcinoma and papilloma) at high incidences after repeated dermal application of low doses** **Tumour development on the skin:** **1 nmol (≈0.3 µg/d):** Mice with skin tumours: 1/24 (4 %) squamous cell papilloma, mean latency: 33 wk; survival: 47 ± 2 wk; 5 lung adenoma **4 nmol (≈1.2 µg/d):** Mice with skin tumours: 19/23 (**83 %**, p<0.001), 16/23 squamous cell carcinoma (70 %) 9/23 squamous cell papilloma (39 %) Mean latency: 28 ± 9 wk; Survival: 46 ± 3 wk; Mice with metastasis in lungs and lymph nodes; 2 adenoma, lung; 5 malignant lymphoma, spleen; 2 malignant lymphoma with multiple organ involvement **8 nmol (≈2.4 µg/d):** Mice with skin tumours: 20/22 (**91 %**, p<0.001), 20/22 squamous cell carcinoma (91 %) 16/22 (73 %) squamous cell papilloma Mean latency: 22 ± 9 wk; Survival: 43 ± 8 wk; Metastasis in lungs; 1 adenoma, lung; 8 malignant lymphoma, spleen; 1 malignant lymphoma with multiple organ involvement	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Control (vehicle): 0/27 no skin tumour; 1 adenoma, lung	
Carcinogenicity study by repeated topical application to the skin no guideline Dose group: 1 Control group: 1 30 µg/mouse painting on shaved dorsal mouse skin followed by 6 µg/mouse painting on shaved dorsal mouse skin 1x/wk, for up to 20 weeks Tumour response was recorded weekly for each mouse Complete necropsy, for histology skin tissues were fixed in 10 % phosphate-buffered formalin, paraffin-embedded sections, stained with H&E	**Mouse** aryl hydrocarbon receptor (AhR)-deficient C57BL/6J (mixed 129/SV and C57BL/6J; 1st generation of AhR+/-mice backcrossed) Female (6-8 wk old) AhR-/-: 15 AhR+/+: 17 Control: Acetone, animal number not given	**DB[a,l]P** Purity: commercial grade Vehicle: Acetone	30 µg (1x) followed by 6 µg 1x/wk, for up to 20 weeks AhR+/+: study duration at death/ sacrifice: wk 24 AhR-/-: observation was extended up to 2 years	**Positive** **DB[a,l]P induced significant skin tumours at high incidences after repeated dermal application of low doses to AhR+/+ mice** **In AhR-/-mice tumour development was suppressed.** **Tumour development on the skin:** **1.2 mg/kg bw (1x) DB[a,l]P; followed by 34 µg/kg bw (1x/wk) for up to 20 wk:** **→Squamous cell papilloma/carcinoma** **AhR+/+ mice:** 17/17, **100 %** wk 24 First skin tumour: After 11 wk Average number of skin tumours/treated mouse: 2.7 ± 1.4 76 % squamous cell papilloma 24 % squamous cell carcinomas **Tumour development on the skin:** **→Squamous cell papilloma** (no malignant skin tumours) **AhR-/-mice:** 5/15, **33 %** wk 24 First skin tumour: After 21 wk Average number of skin tumours/treated mouse:	Nakatsuru et al. 2004

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				0.46 ± 0.83 All 5 skin tumours were squamous cell papilloma No further increase in tumour development on the skin during the follow-up period of up to 2 years **Control (vehicle):** no data available	
Dermal initiation–promotion studies (skin painting)					
Dermal initiation–promotion study **(skin painting)** no guideline Method, study protocol: to identify chemicals with promoting potential Either single or multiple topical sub-carcinogenic dose/s of a chemical is/are first applied to the back of the skin (initiation) followed by repeated topical applications of one or more chemicals (promotion) Skin is monitored for tumour development	**Mouse** Swiss albino Ha/ICR/Mil Female (7–8 wk) 30/group Controls: (1) 2.5 % croton oil in acetone, 30 (2) p-dioxane, 20	**DB[a,l]P** purified by chromatography followed by recrystallization Vehicle: p-dioxane	25 µg per animal (0.1 % solution, 10 x/ 20 days), 8 days after initiation followed promotion with 2.5 % (2.3 mg) croton oil in acetone (volume not spec.) 3 x/wk Control: (1) 2.5 % croton oil in acetone (2) p-dioxane Study terminated after 6 months	**Positive** **Significant tumour-initiating activity on mouse skin after repeated administration of 25 µg DB[a,l]P/mouse** and promotion with 2.5 % croton oil in acetone **Tumour development on the skin:** **25 µg (0.14 mg/kg bw/d) DB[a,l]P:** → **Squamous cell papilloma** ‘mo’: Months after treatment Number of mice with squamous cell papilloma (in %) / number of mice alive: **2 mo: 3 (10.0 %) / 30** **3 mo: 11 (37.9 %) / 29** **4 mo: 15 (53.6 %) / 28** **5 mo: 17 (60.7 %) / 28** **6 mo: 18 (64.3 %) / 28** mean latency period: 94 days	Hoffmann and Wynder 1966

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
9 synthesized and/or highly purified hexacyclic aromatic hydrocarbons were tested Dose group: 1 Control group: 2 Animals monitored weekly by palpation for tumour development, killed when tumours persisted for 4-5 weeks Complete autopsy, histology of skin and tissues suggestive of neoplasia				**Control (promoter):** 2/30 (7 %) squamous cell papilloma **Control (vehicle only):** 0/20 no skin tumours	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Comparative studies of tumour-initiating activity of several dibenzo[a]pyrenes	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis Female (8 wk old) 24/group Control: 100 µL dioxane/DMSO, 23	**DB[a,l]P** Purity: >99 % Vehicle: Dioxane/ DMSO (75:25)	242 µg (800nmol) in 100 µL dioxane/ DMSO (75:25), 1×, followed 3 wk later by promotion with 2.6 µg TPA in 100 µL acetone, 2×/wk Vehicle: Dioxane/ DMSO Study terminated	**Positive** **Significant tumour-initiating activity on mouse skin after a single dose of 242 µg DB[a,l]P/mouse** and promotion with 2.6 µg TPA **Tumour development on the skin: A**fter single initiating dose of **242 µg (9.6 mg/kg bw) DB[a,l]P**: → **Squamous cell papilloma** First skin tumour: A**fter 5 weeks,** tumour-bearing mice: 40 % <u>After wk 18:</u> 92 % with papilloma <u>After 25 weeks:</u>	Cavalieri et al. 1989

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Dose group: 1 Control group: 1 Animals were killed after the 25th week of promotion Number of skin tumours was charted weekly Complete necropsies were performed, and skin tissues were fixed in 10 % buffered formalin			after 25 wk	No. of mice alive: 24/24 No. of squamous cell papilloma: 92 No. of tumour-bearing mice (in %): 22/24 (92 %) No. of squamous cell papilloma /mouse (calculated by dividing the total number of papilloma by the total number of treated mice per group): 3.8 **Control (vehicle only):** First skin tumour: **After 20 weeks** <u>After 25 weeks:</u> No. of mice alive: 23/23 No. of squamous cell papilloma: 2 No. of tumour-bearing mice (in %): 2/23 (9 %) No. of squamous cell papilloma/treated mouse: 0.1	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Initiation: painting on shaved dorsal mouse skin (1x), promotion	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis Female (8 wk old) 24/group Control: 100 µL acetone, 24	**DB[a,l]P** Purity: >99 %, 161-162 °C, HPLC Vehicle: Acetone	33.3, 100 or 300 nmol (10.1, 30.2, 90.7 µg) in 100 µL acetone 1x, followed 1 wk later by promotion with TPA (3.2 nmol, ≈2.0 µg) in 100 µL acetone, 2×/wk Due to severity	**Positive** **Significant tumour-initiating activity on mouse skin after a low single dose of DB[a,l]P/mouse** and promotion with 2.0 µg TPA **Tumour development on the skin: A**fter single initiating dose of: **10.1 µg (0.4 mg/kg bw):** **→ Squamous cell papilloma** First tumour: After 8 wk <u>After 16 weeks:</u> No. of tumour-bearing mice (in %): 23/24 (96 %)	Cavalieri et al. 1991

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
with TPA 2x/wk Dose group: 3 Control group: 1 Number of skin tumours was charted weekly Mice were killed 15 wk after initiation Complete necropsies were performed, and skin tissues were fixed in 10 % buffered formalin (Tumours were predominantly papilloma. Very few (1-4/group) appeared to be carcinomas, but they were not histologically verified.)			of reaction promotion was stopped until the 4th wk, continued for 11 wk Study terminated after 16 wk	No. of tumours/treated mouse: 6.75 **30.2 µg (1.2 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 4 wk <u>After 16 weeks:</u> No. of tumour-bearing mice (in %): 22/24 (92 %) No. of tumours/treated mouse: 7.92 **90.7 µg (3.6 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 4 wk <u>After 16 weeks:</u> No. of tumour-bearing mice (in %): 24/24 (100 %) No. of tumours/treated mouse: 8.5 **Control (vehicle only):** 0/24, no skin tumours	
Dermal initiation–promotion study **(skin painting)** no guideline	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis	**DB[a,l]P** Purity: >99 %, 161-162 °C, HPLC	4, 20 or 100 nmol (1.2, 6.0 or 30.2 µg) in 100 µL acetone 1x Due to severity	**Positive** **Significant tumour-initiating activity on mouse skin after a low single dose of DB[a,l]P/mouse** and promotion with 2.0 µg TPA **Tumour development on the skin:** After single initiating dose of:	Cavalieri et al. 1991

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
(for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 3 Control group: 1 Initiation: painting on shaved dorsal mouse skin (1x), promotion with TPA 2x/wk Number of skin tumours was charted weekly Mice were killed 27 wk after initiation Complete necropsies were performed, and skin tissues were fixed in 10 % buffered formalin (Tumours were predominantly papilloma, but included the histology verified squamous cell carcinoma.)	Female (8 wk old) 24/group Control: 100 µL acetone, 24	Vehicle: Acetone	of reaction promotion was delayed until the 3th exp. wk followed by promotion with TPA (3.2 nmol, ≈2.0 µg) in 100 µL acetone, 2×/wk for up to 24 wk Study terminated after 27 wk	**1.2 µg (0.048 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 10 wk After 27 weeks: No. of tumour-bearing mice (in %): 22/24 (92 %) No. of tumours/treated mouse: 6.96 Verified squamous cell carcinoma: 2 **6.0 µg (0.24 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 10 wk After 27 weeks: No. of tumour-bearing mice (in %): 20/24 (83 %) No. of tumours/treated mouse: 5.29 Verified squamous cell carcinoma: 3 **30.2 µg (1.2 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 4 wk After 27 weeks: No. of tumour-bearing mice (in %): 20/24 (83 %) No. of tumours/treated mouse: 3.29 Verified squamous cell carcinoma: 2	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Control (vehicle only): 0/24, no skin tumours	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 2 Control group: 1 Initiation: painting on shaved dorsal mouse skin (1x), promotion with TPA 2x/wk Number of skin tumours was charted weekly Mice were killed 27 wk after initiation Complete necropsies were performed, and skin tissues were fixed in 10 % buffered formalin	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis Female (8 wk old) 24/group Control: 100 µL acetone, 24	**DB[a,l]P** Purity: >99 %, 161-162 °C, HPLC Vehicle: Acetone	0.25 or 1.00 nmol (75.5 or 302 ng) in 100 µL acetone, 1x followed 1 wk later by promotion with TPA 2.16 nmol, (≈1.3 µg) in 100 µL acetone, 2×/wk study terminated after 27 weeks	**Positive** **Significant tumour-initiating activity on mouse skin after a low single dose of DB[a,l]P/mouse** and promotion with 1.3 µg TPA **Tumour development on the skin:** (Tumours were all squamous cell papilloma, with the exception of 2 carcinomas in the 1.00 nmol group.) **A**fter single initiating dose of: **75.5 ng (3.0 µg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 10 wk <u>After 27 weeks:</u> No. of tumour-bearing mice: ~30 % No. of tumour/treated mouse: 0.8 **302 ng (12 µg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 5 wk <u>After 27 weeks:</u> No. of tumour-bearing mice: ~80 % No. of tumour/treated mouse: 2.8 Verified squamous cell carcinoma: 2	Higginbotham et al. 1993

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Control (vehicle only): 0/24, no skin tumours	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 4 Control group: 1 Initiation: painting on shaved dorsal mouse skin (10x) promotion with TPA 3x/wk for up to 20 wk Number of skin tumours was charted weekly No histology data	**Mouse** CD-1 Female (28-35 days old) 19-20/group Control: 100 µL acetone, 20	**DB[a,l]P** Purity: commercial grading Vehicle: Acetone	1, 4, 10 or 25 nmol (0.3, 1.2, 3.0 or 7.6 µg) in 100 µL acetone, over 10 days followed by 2.5 µg TPA in 100 µL acetone 3x/wk, for 20 wk study was terminated after promotion for up to 20 wk	**Positive** **Significant tumour-initiating activity on mouse skin after repeated applications of low dose DB[a,l]P/mouse** and promotion with 2.5 µg TPA **Tumour development on the skin:** (→macroscopic findings as nodules) After repeated applications (over 10 days) of: **1 nmol (0.3 µg; ≈0.012 mg/kg bw/d):** First skin tumour: After 6 wk No. of tumour-bearing mice (in %): 18/19 (95 %) No. of tumour/treated mouse: 5.0 **4 nmol (1.2 µg; ≈0.048 mg/kg bw/d):** First skin tumour: After 4 wk No. of tumour-bearing mice (in %): 20/20 (100 %) No. of tumour/treated mouse: 17.8 **10 nmol (3.0 µg; ≈0.12 mg/kg bw/d):** First skin tumour: No data No. of tumour-bearing mice (in %): 18/20 (90 %) No. of tumour/treated mouse: 11.3	LaVoie et al. 1993

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				25 nmol (7.6 µg; ≈0.3 mg/kg bw/d): First skin tumour: No data No. of tumour-bearing mice (in %): 20/20 (100 %) No. of tumour/treated mouse: 15.0 **Control (vehicle only):** No. of tumour-bearing mice (in %): 3/20 (15 %) No. of tumour/treated mouse: 0.15	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 3 Control group: none Initiation: painting on shaved dorsal mouse skin (1x) promotion with TPA 2x/wk Tumours was charted weekly	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis Female (8 wk old) 23-25/group No controls	**DB[a,l]P** Purity: multi-step synthesis and purification by chromato-graphy Vehicle: Acetone	1.33, 4 or 12 nmol (0.4, 1.2, or 3.6 µg) in 100 µL acetone followed 1 wk later by promotion with TPA (1.62 nmol ≈1.0 µg) in 100 µL acetone, 2×/wk for 28 wk study terminated after 29 weeks	**Positive** **Significant tumour-initiating activity on mouse skin after a low single dose of DB[a,l]P/mouse** and promotion with 1.0 µg TPA **Tumour development on the skin: A**fter single initiating dose of: **1.33 nmol (0.016 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 10 wk No. of tumour-bearing mice (in %): 16/23 (70 %) No. of tumour/treated mouse: 5.22 Verified squamous cell carcinoma: 2 carcinoma in 2 mice **4 nmol (0.048 mg/kg bw):** **→ Squamous cell papilloma**	Gill et al. 1994

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Mice were killed 29 wk after initiation Complete necropsies were performed, and tissues were fixed in 10 % buffered formalin, histology of tumours				First skin tumour: After 8 wk No. of tumour-bearing mice (in %): 19/23 (83 %) No. of tumour/treated mouse: 7.09 **12 nmol (0.14 mg/kg bw):** **→ Squamous cell papilloma** First skin tumour: After 6 wk No. of tumour-bearing mice (in %): 23/25 (92 %) No. of tumour/treated mouse: 9.28 Verified squamous cell carcinoma: 7 carcinoma in 5 mice **No controls**	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 1 Control group: 1	**Mouse** outbred NMRI Female (7 wk old) 16/group Control: 100 µL acetone, 16	**DB[a,l]P** Purity: multistep synthesis and purification by chromato-graphy, ≥99.7 % Vehicle: acetone	40 nmol (12 µg) in 100 µL acetone followed 1 wk later by promotion with TPA (10 nmol, ≈6.2 µg) in 100 µL acetone, 2×/wk for 30 wk study terminated after 30 weeks	**Positive** **Significant tumour-initiating activity on mouse skin after a single dose of DB[a,l]P/mouse** and promotion with 6.2 µg TPA **Tumour development on the skin:** (→macroscopic findings as nodules) After single initiating dose of: **40 nmol (0.48 mg/kg bw):** First skin tumour: After 8 wk <u>After 18 wk:</u>	Luch et al. 1999

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Initiation: painting on shaved dorsal mouse skin (1x) promotion with TPA 2x/wk Monitoring weekly by palpation for tumour development Mice were scored when nodules reached ≥1 mm in diameter Surviving mice were killed after 30 weeks No histology				No. of tumour-bearing mice (in %): 15/16 (94 %) No. of tumour/treated mouse: 6.5 **Control (vehicle only):** 0/16, no skin tumours	
Dermal initiation–promotion study **(skin painting)** no guideline (for general method and study protocol s. reference Hoffmann and Wynder (1966)) Dose group: 1 Control group: 1 Initiation: painting on shaved dorsal mouse skin (1x)	**Mouse** SENCAR derived from SENsitivity to CARcinogenesis Female (6-7 wk old) 35/group Control: 200 µL toluene, 10	**DB[a,l]P** Purity: commercial grade Vehicle: Acetone	2 nmol (0.6 µg) in 200 µL acetone followed 2 wk later by promotion with TPA (1 µg) in 200 µL acetone, 2×/wk for 25 wk Study terminated after 25 weeks	**Positive** **Significant tumour-initiating activity on mouse skin after a single dose of DB[a,l]P/mouse** and promotion with 1.0 µg TPA **Tumour development on the skin:** After single initiating dose of: **2 nmol (0.024 mg/kg bw):** → **Squamous cell papilloma** First skin tumour: After 6 wk Skin tumour after: 8 wk: 63 % 10 wk: 95 % 15 wk: 100 %	Marston et al. 2001

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
promotion with TPA 2x/wk Monitoring weekly by palpation for skin tumour development Necropsy, histology of tumours				<u>After 25 wk (study termination):</u> Mortality: 5/35 No. of tumour-bearing mice (in %): 30/30 (100 %) No. of tumour/tumour-bearing mouse: 8.1 No. of tumour/treated mouse: 7.87 **Control (vehicle toluene):** Mortality: 1/10 No. of tumour-bearing mice (in %): 1/9 (11 %) No. of tumour/treated mouse: 0.25	

4.10.2 Human information

No case reports or epidemiological studies on the significance of DB[a,l]P exposure to man are available. DB[a,l]P has been detected in several environmental soil and sediment samples, in cigarette smoke condensate and in particulate matter formed during combustion of smoky coal. However, coal-tar and other materials which are known to be carcinogenic to man may contain DB[a,l]P. It is also contained in certain consumer articles, such as toys, tool handles, bicycle grips, shoes, sport equipment (BfR 2009; UBA 2010; Wennemer 2009; Hutzler et al. 2011). The possible contribution of PAH from environmental sources to the overall carcinogenic risk to man is discussed in the general remarks in IARC (2010).

Individual PAH are found in the environment not in isolation but as components of highly complex mixtures of chemicals. PAH are very widespread environmental contaminants, because they are formed during incomplete combustion of materials such as coal, oil, gas, wood, or garbage or during pyrolysis of other organic material, such as tobacco or charbroiled meat. Data on the carcinogenicity of PAH in humans are available only for mixtures containing PAH. Evidence that mixtures of PAH are carcinogenic to humans is primarily derived from occupational studies of workers following inhalation and dermal exposure, especially from coke oven workers and aluminium smelters. These data clearly showed lung and bladder cancer in exposed workers. Skin cancer in man is well known to occur following exposure to poorly refined lubricating and cutting oils. No data were located regarding cancer in humans following inhalation or dermal exposure of individual PAH compounds. It is difficult to ascertain the carcinogenicity of the single component PAH in these mixtures because of the presence of other carcinogenic substances in the mixtures. In 2005, IARC re-evaluated the PAH. Although certain occupations with high PAH exposure (e.g. coal gasification process and coke production) were classified as carcinogenic in humans, the role of individual PAH could not be defined (IARC 2010).

4.10.3 Other relevant information

Table 20: Results from carcinogenicity studies in rats, injections in mammary gland

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Carcinogenicity study by injections in mammary gland no guideline Dose group: 1 Control group: 2 Single injection in each mammary gland (8) Animals monitored weekly by palpation for tumour development, killed when tumours ≥2 cm in diameter After 15 wk survivors were sacrificed Complete necropsy Mammary tumours, as well as other grossly abnormal tissues were fixed in 10 % buffered formalin, sectioned and stained with H&E for microscopy	**Rat** Sprague-Dawley Female (8 wk old) 19/group Control: (1) 100 µL trioctanoin, 21 (2) No treatment, 20	**DB[a,l]P** >99 % by HPLC; recrys-tallised from xylenes	Single injection of 4 µmol (1.2 mg) dissolved in 100 µL trioctanoin per mammary gland (8 glands total dose, 9.6 mg) Control: 100 µL trioctanoin Study terminated after 15 wk	**Positive** **DB[a,l]P induced benign and malignant tumours in the mammary gland after single injection of 38.4 mg/kg bw** <u>Mortality:</u> 10/19 (53 %) died in the first 9 wk after treatment as a result of the toxic effects of the compound. The animals showed sign of distress and cachexia. At necropsy these animals had little or no body fat, their lungs were almost white and the skin lining was slightly oedematous and had a bright yellow cast. Due to poor conditions of the rats in this group and palpable nodules in the mammary gland the remaining animals were sacrificed in the 15th week. →**Tumour development in the mammary gland:** After single injection in each gland: First tumour: After 7 wk (survivors) <u>After 12 wk:</u> No. of tumour-bearing rats (in %): 7/9 (77.8 %); mean tumour latency: 9 ± 1 wk <u>After 14 wk:</u> No. of tumour-bearing rats (in %): 9/9 (100 %); mammary adenocarcinoma: 8/9 (89 %) No. of tumour/tumour-bearing rats: 3.8;	Cavalieri et al. 1989

				fibrosarcoma: 7/9 (78 %) No. of tumour/tumour-bearing rats: 2.1 **→Tumour development on the skin:** After single injection in each gland: → Squamous cell carcinoma No. of tumour-bearing rats (in %): 8/9 (89 %) No. of tumour/tumour-bearing rats: 2.4 **Control (no treatment):** 2/20 (10 %) mammary epithelial tumours (1 adeno-fibroma, 1 adenocarcinoma) **Control (vehicle):** 0/21 (0 %) no tumour in mammary gland	
Carcinogenicity study by injections in mammary glands no guideline Dose group: 2 Control group: 1 Single injections in each mammary gland, under the nipple region of the 2nd, 3rd, 4th, 5th mammary gland on the left and right site Animals monitored weekly by	**Rat** Sprague-Dawley Female (8 wk old) 19-20/group Control: 50 µL trioctanoin, 18	**DB[a,l]P** >99 % by HPLC; re-cry-stallised from xy-lenes	Single injection of 0.25 or 1 µmol (75.6 or 302 µg) dissolved in 50 µL trioctanoin per mammary gland (8 glands) total 605 µg/rat or 2.4 mg/rat Control: 50 µL trioctanoin per mammary gland (8 glands)	**Positive** **DB[a,l]P induced in rats malignant tumours in the mammary gland after single injection** **Tumour development in the mammary gland:** After single initiating dose of: **0.25 µmol (2.4 mg/kg bw)**: **→ Mammary adenocarcinoma** Mean survival: 20 ± 2 wk No. of tumour-bearing rats (in %): 20/20 (100 %); mean tumour latency: 14 ± 2 wk No. of tumour/tumour-bearing rats: 6.3 **→ Fibrosarcoma** No. of tumour-bearing rats (in %): 4/20 (20 %)	Cavalieri et al. 1991

palpation for tumour development, killed when tumours ≥2 cm in diameter After 24 wk survivors were sacrificed Complete necropsy Mammary tumours, as well as other grossly abnormal tissues were fixed in 10 % buffered formalin, sectioned and stained with H&E for microscopy			Study terminated after 24 wk	No. of tumour/tumour-bearing rats: 1.3 **1.0 µmol (9.6 mg/kg bw):** **→ Mammary adenocarcinoma** Mean survival: 17 ± 2 wk No. of tumour-bearing rats (in %): 19/19 (100 %) Mean tumour latency: 11 ± 2 wk No. of tumour/tumour-bearing rats: 9.1 **→ Fibrosarcoma** No. of tumour-bearing rats (in %): 14/19 (74 %) No. of tumour/tumour-bearing rats:2.4 **Tumour development in the skin:** After single initiating dose of: **0.25 µmol (2.4 mg/kg bw)**: 1 squamous cell carcinoma of the skin **1.0 µmol (9.6 mg/kg bw):** 1 squamous cell carcinoma of the skin **Control (vehicle):** Mean survival: 24 ± 0 wk No. of tumour-bearing rats, (in %): 1/18 (6 %) mammary adenofibroma Mean tumour latency: 22 ± 0 wk	

Table 21: Results from carcinogenicity studies in mice

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Carcinogenicity study by subcutaneous (s.c.) administration					
Carcinogenicity study by s.c. administration no guideline Dose group: 1 Control group: 1 2 injections of 0.6 mg in 0.2 mL neutral sterile olive oil 1 x/month, 1 month elapsing between each injection After 2 months 3th injection to a few mice which had no strong fibrous reaction at the injection site	**Mouse** XVII nc/ZE (3-4 months old) 12 males and 12 females Control: 0.2 mL neutral sterile olive oil, 500 (sex not given)	**DB[a,l]P** Purity: not specified	0.6 mg in 0.2 mL neutral sterile olive oil 2x (1x/month), 1 additional injection 2 months later (only to mice without strong fibrous reaction on injection site, no number/sex is given) Study terminated after 195 days (males), 217 days (females)	**Positive** **DB[a,l]P induced local sarcoma at the injection site after single subcutaneous injection of 0.6 mg per month (total of 3 injections)** **Development of local sarcoma:** After injection of (total 1.8 mg) **51.4 mg/kg bw (male mice)**: **12/12 (100 %)** mean tumour latency: 130 days **60 mg/kg bw (female mice): 12/12 (100 %)** mean tumour latency: 113 days Control (vehicle): no skin tumours in over 500 animals (sex not given) treated with the solvent	Lacassagne et al. 1968
Carcinogenicity study by intraperitonaeal (i.p.) injection					
Carcinogenicity study by single i.p. injections no guideline	**Mouse**	**DB[a,l]P** Purity: pure analytical	Single injection of <u>Test 1:</u> 0.3, 1.5, 3.0, or 6 mg/kg bw in tricaprylin	**Positive** **DB[a,l]P induced significant numbers of lung adenomas in a dose-dependent manner**	Prahalad et al. 1997

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Dose group: 7 Control group: 2 Single injections as a uniform suspension in triaprylin Animals weights were recorded prior to injection and at time of sacrifice →tumourigenicity study, examination of the tumour development in lungs (test 1) → survival study (test 2) Termination: 8 months after treatment 30-35 animals/dose group Gross necropsy; lungs were removed and examined for presence of tumours; lungs were fixed in 10 % neutral buffered formalin, prepared for microscopy	A/J mouse lung Males (5-6 wk old, ~20 g) Tumouri-genicity study: 30-35/dose group Survival study: 5/dose group Control: (1) Tricaprylin, 30 (2) Positive control: 1000 mg/kg bw urethane, 30	grading	<u>Test 2:</u> 12, 18, or 24 mg/kg bw in tricaprylin Vehicle: tricaprylin Study duration: 8 months	<u>Tumourigenicity study:</u> **→Tumour development in the lung:** **0.3 mg/kg bw:** No. of tumour-bearing mice (in %): 14/33 **(43 %)** No. of average lung adenoma/treated mouse: 0.42 ± 0.56 **1.5 mg/kg bw:** No. of tumour-bearing mice (in %): 33/34 **(97 %)** No. of average lung adenoma/treated mouse: 4.30 ± 2.86 **3.0 mg/kg bw:** No. of tumour-bearing mice, (in %): 35/35 **(100 %)** No of average lung adenoma/treated mouse: 7.50 ± 3.79 **6.0 mg/kg bw:** No. of tumour-bearing mice, (in %): 30/30 **(100 %)** No. of average lung adenoma/treated mouse: 16.1 ± 7.26 **Liver:** Mild increase in severity and incidence of hepatocellular necrosis and inflammation, not dose-related	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Vehicle Control: No. of tumour-bearing mice (in %): 15/30 (50 %) No. of average lung adenoma/treated mouse: 0.67 ± 0.80 **Positive Control** (urethane, 1000 mg/kg bw): No. of tumour-bearing mice (in %): 30/30 **(100 %)** No. of average lung adenoma/treated mouse: 31.4 ± 8.94 <u>Survival study:</u> Up to 24 mg/kg bw: No effect on survival 12 or 18 mg/kg bw: No significantly effect on bw; no more data **24 mg/kg bw:** 22 % bw loss; development of lung adenoma: 5/5 (100 %); No. of average lung adenoma/treated mouse: 36.67 ± 10.64	
Carcinogenicity study by i.p. injections no guideline Dose group: 2 Control group: 3	**Mouse** New-born Crl:CD®-1(ICR)BR Male and female: both about 70-100 3 control groups:	**DB[a,l]P** Purity: synthesized	i.p. injections on days: 1, 8 and 15 of life total dose: 400 or 40 nmol (121 or 12.1 µg) dissolved in 80 µL DMSO Total dose of 121 µg was given as: Day 1: 1/8 dose (15.2 µg) in 10 µL DMSO; Day 8:	**Positive** **DB[a,l]P induced significant number of tumours in the lung, liver and in nine other organs of newborn mice treated on days 1, 8 and 15 of life** **→Tumour development in the lung** **Among all: 98.1 % adenoma; 1.2 % adenocarcinoma** **400 nmol (total 121 µg):** different	Platt et al. 2004

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Repeated (3x) i.p. injections of new-born mice on days 1, 8 and 15 of life with DB[a,l]P dissolved in DMSO At 3–4 wk of age animals were weaned and separated by sex Study termination at 55 ± 1 wk after onset Gross necropsy Lungs, liver and various other organs were collected, fixed in aqueous formaldehyde solution (4 %, pH 7), prepared for microscopy, H&E and van Gieson trichrome staining	(1) No treatment,75 (2) DMSO, 76 (3) 400 nmol anthracene, 105		1/4 dose (30.2 µg) in 20 µL DMSO; Day 15: 5/8 dose (75.6 µg) in 50 µL DMSO Total dose of 12.1 µg was given as: Day 1: 1/8 dose (1.52 µg) in 10 µL DMSO; Day 8: 1/4 dose (3.02 µg) in 20 µL DMSO; Day 15: 5/8 dose (7.56 µg) in 50 µL DMSO Control: (2) Days 1 /8 /15: 10 /20 / 50 µL, total 80 µL DMSO (3) 400 nmol anthracene Termination of the study: 55 ± 1 wk	intensity of effects in the individual litters; high mortality from: 5 litters: m: 17/25, f: 14/25 reached an age of 17 wk, and a 6th (same) litter: m: 4/5, f: 3/6 reached an age of 39 wk <u>After 17 wk:</u> males: tumour incidence: 41.2 %, average tumours/treated mouse: 0.65 ± 0.21 females: tumour incidence: 35.7 %, average tumours/treated mouse: 0.57 ± 0.29 <u>After 39 wk:</u> males: tumour incidence: 100 %, average number of tumours/treated mouse: 15.25 ± 4.70 females: tumour incidence: 100 %, average number of tumours/treated mouse: 13.67 ± 1.45 **40 nmol (total 12.1 µg): After 55 ± 1 wk:** m: 33/36, f: 19/30 reached termination at 55 wk males: tumour incidence: 84.8 %, average number of tumours/treated mouse: 2.85 ± 0.44 females: tumour incidence: 89.5 %, average number of tumours/treated mouse: 2.95 ± 0.67	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				Control, tumour incidences after 55 ± 1 wk: (no treatment; DMSO; anthracene) males: 31.4 %; 25.0 %; 21.4 % females: 37.0 %; 10.0 %; 38.7 % **→Tumour development in the liver** **Among all: 89.0 % adenoma; 4.6 % carcinoma:** **400 nmol (total 121 µg):** After 17 wk : males: tumour incidence: 35.3 %, average tumours/treated mouse: 1.0 ± 0.38 females: tumour incidence:14.3 %, average tumours/treated mouse: 0.21 ± 0.15 After 39 wk: males: tumour incidence: 25 %, average tumours/treated mouse: 0.25 ± 0.25 females: tumour incidence: 0 % **40 nmol (total 12.1 µg): 55 ± 1 wk:** males: tumour incidence: 84.8 %, average tumours/treated mouse: 5.67 ± 0.86; females: tumour incidence: 10.5 %, average tumours/treated mouse: 0.11 ± 0.07 **Control,** tumour incidences after	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				55 ± 1 wk (no treatment; DMSO; anthracene): males/females: 0 % tumours/treated mouse: 0.50 ± 0.17 <u>After 39 wk:</u> males: tumour incidence: 0 % females: tumour incidence: 33.3 %, average tumours/treated mouse: 1.0 ± 1.0 **40 nmol (12.1 µg): 55 ± 1 wk:** males: tumour incidence: 30.3 %, average tumours/treated mouse: 0.58 ± 0.17 female: tumour incidence: 47.4 %, average tumours/treated mouse: 0.53 ± 0.14 **Control,** tumour incidences after 55 ± 1 wk (no treatment; DMSO; anthracene): males/females: 0 %	

4.10.4 Summary and discussion of carcinogenicity

DB[a,l]P is possibly carcinogenic to humans. Classification as carcinogen is largely based on animal evidence. There is sufficient evidence on carcinogenicity of DB[a,l]P from animal studies.

Evidence from animal experiments

The carcinogenic potential of DB[a,l]P has been studied in three rodent species (mice, rats and hamster) under different protocols.

DB[a,l]P was tested for carcinogenicity in two oral studies, in one study by repeated oral (gavage) application to female mice (two strains: wild-type and P450 1B1-null) (Buters et al. 2002; see Table18) and in hamster with applications on the tongue (Schwartz et al. 2004; see Table 18), in five studies with topic application on the mouse skin (one with single and four with repeated application; see Table 19), in a study by subcutaneous administration in mice (Lacassagne et al. 1968; see Table 21), in nine dermal tumour initiation–promotion studies (skin painting) in different mouse strains (see Table 19), by intraperitoneal administration to male A/J mice (Prahalad et al. 1997; see Table 21) and to new-born mice (Platt et al. 2004; see Table 21), and two studies by single injection in the mammary glands of rats (Cavalier1 et al. 1989, 1991; see Table 20). DB[a,l]P exhibited significant carcinogenic activity in all of these studies.

Repeated oral application of low dose of DB[a,l]P (approx. 1.07 mg/kg bw/d for up to 3 weeks) by gavage to mice of two strains induced benign and malignant tumours in various organs, e.g. ovaries, uterus, lymphoid tissues and skin; and oral squamous cell carcinoma after repeated applications of low doses (3 µg) on the tongue of hamsters.
Repeated dermal application of DB[a,l]P of rather low concentrations caused benign and malignant skin tumours (squamous cell papilloma and carcinoma) at high incidences in mice of both sexes. A dose-response relationship for tumour induction was observed in these studies with repeated dermal application of DB[a,l]P. In mice the lowest dose which produced squamous cell papilloma and carcinoma in females was approximately 1.2 µg/d. Nineteen of 23 animals developed skin tumours within 28 weeks following dermal application of this dose in acetone as vehicle. These tumours progressed rapidly in size and killed the host within 46 weeks. In studies with higher doses shorter latency periods were observed for tumour development in the skin of mice. In addition mice with metastasis in lungs and lymph nodes were found.

Local sarcoma at the site of administration was observed in mice of both sexes following three subcutaneous injections of 0.6 mg (a total of approx. 51.4 mg/kg bw in males and 60 mg/kg bw in females) DB[a,l]P.

Single intraperitoneal injection of 0.3 mg/kg bw DB[a,l]P and higher to adult male mice induced numbers of lung adenomas in a dose-dependent manner; and intraperitoneal injections of new-born mice on three days of life (1, 8 and 15) with DB[a,l]P (total 12.1 µg and higher) caused benign and malignant lung tumours in both sexes of high incidences. DB[a,l]P also caused high incidences of benign and malignant liver tumours and tumours in other organs. The occurrence of these tumours clearly emphasizes the high tumorigenic potency of DB[a,l]P. It was noted that DB[a,l]P-induced lung tumours develop slowly compared to liver tumours. Seventeen weeks into the experiment, the incidence of pulmonary tumours was about as low as that in control animals but it rose dramatically thereafter. At week 35, all mice (100 %) were diagnosed with lung tumours.

Doses of 2.4 mg/kg bw and higher DB[a,l]P administered by single intra-mammary injection caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in all treated animals and squamous cell carcinoma in the skin of 89 % of female rats after 14 weeks.

In dermal initiation-promotion studies (skin painting studies) significant tumour-initiating activity of DB[a,l]P was demonstrated in four mouse strains. Squamous cell papilloma began appearing 5 weeks after initiation with DB[a,l]P of low doses and after only two weeks of promotion.

Overall the data base have shown that DB[a,l]P caused benign and malignant tumours in mice and rats at multiple tissue sites, and by different routes of exposure, and in hamster after repeated applications on the tongue.

Human data

No data are available in humans exposed to pure DB[a,l]P.

There are a number of epidemiologic and mortality studies that show increased incidences of cancer in humans exposed to mixtures of PAH (IARC 2006; US EPA 1984; WHO 1987, 1998, 2000, 2003; Armstrong et al. 1994, 2003, 2004, 2009; Boffetta et al. 1997; Bosetti et al. 2007; Costantino et al. 1995; Mastrangelo et al. 1996; Moolgavkar et al. 1998). Most of the PAH have been shown to be initiators of skin and lung cancer (IARC 1983, 2010). This feature was also provided for DB[a,l]P in mice.

Additional information on classification of the structurally similar substances DB[a,i]P and DB[a,h]P

The classification proposal for DB[a,i]P and DB[a,h)]P as Carc. 1B agreed by RAC at its 41th meeting (June 2017) is based on animal experiments in carcinogenicity studies. The results provide sufficient evidence for induction of carcinogenicity in animals (e.g. responses in multiple species and both sexes as well as at different sites with various routes of exposure).

The classification proposals are supported by structural similarity to known carcinogens (B[a]P and chrysene: Carc. 1B).

Germ cell mutagenicity data

DB[a,l]P induces genotoxic effects in vitro and in vivo. A classification as Category 2 mutagen, H341 is proposed in accordance with CLP Regulation due to the positive results of the only guideline compliant tests (in vitro gene mutation test in bacteria and in proliferated cells) combined with a read-across approach to the germ cell mutagens B[a]P and CHR. For more details see Chapter ‘4.9 Germ cell mutagenicity (Mutagenicity)’.

Mode of action

No species-specific mode of action for DB[a,l]P carcinogenesis was identified.

According to today’s state of knowledge the potential mechanisms behind chemical carcinogenesis are several highly complex genotoxic events (mutations), altered gene expression at the transcriptional translational, posttranslational levels (epigenetic events) and altered cell survival (proliferation and apoptosis) (Hanahan and Weinberg 2000).

PAH are a class of chemical carcinogens which undergo metabolic transformations to yield reactive intermediates which bind covalently to DNA, RNA and proteins (Miller et al. 1980; Miller 1970, Boström et al. 2002). The diol epoxide pathway is the most widely accepted pathway of PAH activation to yield DNA adducts (Xue and Warshawsky 2005; Chen et al. 2012). The metabolic activation of PAH occurs in the same way to produce a diol epoxide irrespective of the molecular structure and the different steric conditions at the reactive centres. The pathway is catalysed mainly by several enzymes such as CYPs and epoxide hydrolases. Metabolic activation finally leads to the formation of electrophilic diolepoxides, which belong to the most potent mutagens reported so far **(**Xue and Warshawsky 2005).

4.10.5 Comparison with criteria

According to the CLP Regulation a substance shall be classified as carcinogenic if:

Category 1A carcinogen

'It is known to have carcinogenic potential for humans; classification is largely based on human evidence.'

DB[a,l]P is possibly carcinogenic to humans. The available human data do not allow an allocation of PAH-related carcinogenicity to a single PAH. Therefore classification as Category 1A carcinogen is not appropriate.

Category 1B carcinogen

'It is presumed to have carcinogenic potential for humans; classification is largely based on animal evidence.'

This category depends on the strength of evidence, which consists of animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity. This means a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in

(a) two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols;

(b) in both sexes of a single species;

(c) occurrence of malignant neoplasm to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

In comparison to the given criteria for CLP Regulation DB[a,l]P fulfils the criteria for Category 1B carcinogen with regard to:

There is sufficient evidence of carcinogenicity from studies in experimental animals. In this review of the data related to DB[a,l]P-induced cancer sufficient evidence is available that DB[a,l]P is carcinogenic when administered to mice, rats, and hamster.

DB[a,l]P caused tumours in mice and rats, at multiple tissue sites, and by different routes of administration. Dermal exposure to DB[a,l]P caused benign or malignant skin tumours (squamous cell papilloma or carcinoma) in mice, and subcutaneous injection caused cancer at the injection site (sarcoma) in mice. Multiple tumour sites (e.g. ovaries, uterus, lymphoid tissues and skin) were observed following oral application of DB[a,l]P by gavage to mice; and oral cavity tumours when DB[a,l]P was applied on the tongue of hamsters. Lung tumours were induced in mice following intraperitoneal injection of DB[a,l]P. In addition to lung tumours, DB[a,l]P induced hepatic tumours and a variety of tumours at other sites when administered intraperitoneally to new-born mice. DB[a,l]P administered by intra-mammary injection has caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in female rats.

Benign and malignant tumours induced by DB[a,l]P have been found in the skin (by topical administration); in lung, liver and other organs (by intraperitoneal administration); also in a number of organs and tissues following oral application; and in the mammary gland (by intra-mammary gland injection). In addition a significant tumour-initiating activity of single small DB[a,l]P doses was demonstrated in four mouse strains by dermal initiation-promotion studies.

Category 2 carcinogen

'It is a suspected human carcinogen, but the evidence is not sufficient for Category 1A or 1B.'

Following consideration would lead to classification as category 2:

(a) the evidence is limited to a single experiment;

(b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies;

(c) the agent increases the incidence only of benign neoplasm or lesions of uncertain neoplastic potential; or

(d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Category 2 is not appropriate, because the criteria are not fulfilled. The evidence is neither limited to a single experiment, nor limited with regard to benign neoplasms, and not limited to only promoting activity.

In conclusion, the available data for carcinogenicity of DB[a,l]P give clear evidence of carcinogenic activity in experimental animals. DB[a,l]P fulfils the criteria for classification and labelling as Category 1B carcinogen, H350 (May cause cancer) according to CLP. The classification in Category 1B is based on animal experiments for which there are sufficient evidence to demonstrate animal carcinogenicity.

A causal relationship has been established between DB[a,l]P and an increased incidence of a combination of benign and malignant tumours in mice and rats, in several studies from different laboratories and under different protocols. Further an increased incidence of tumours in both sexes of mice and in female rats (male rats were not tested) was observed in well-conducted studies. Tumour development was noted by the dermal route of administration of small doses in mice. Significantly increased tumour incidences were also seen in studies using subcutaneous and intraperitoneal injections in mice, and in oral studies in mice and hamster.

This classification is in accordance with the classification system of IARC (2010), who ranked DB[a,l]P in group 2B (possibly carcinogenic to humans).

The calculation of specific concentration limits (SCL) to the carcinogen DB[a,l]P is based on the carcinogenicity study by repeated topical application to the skin of mice by Higginbotham et al. (1993). The data of skin tumour development in female mice were assessed as most suitable for the T25 approach (see Annex I: 'Setting specific concentration limits (SCL) for carcinogens: Determination of the carcinogenic potency of DB[a,l]P').

Conclusion: Specific concentration limits for DB[a,l]P, Category 1B carcinogens

According to the 'Guidelines for setting specific concentration limits for carcinogens' for setting specific concentration limits for carcinogens in Annex I of Directive 67/548/EEC inclusion of potency considerations' (EC 1999) and the 'Guidance on Application of the CLP Criteria' (Version 4.1, June 2015, Section 3.6.2.5) DB[a,l]P belongs to the carcinogens of high potency (T25 value $\leq$ 1 mg/kg bw/d).

On the basis of the guidance (EC 1999) described in Chapters 3 and 4a potency evaluation has been made. From this potency evaluation SCL for carcinogens can be derived.

Category 1B carcinogens showing high potency will normally be given a specific concentration limit an order of magnitude lower (0.01 %) than the generic concentration limits of ingredients of a mixture classified as carcinogen that trigger classification of the mixture of ≥ 0.1% (see EC 1999). In accordance with these guidance Category 1B carcinogens showing high potency (T25 ≤ 1 mg/kg bw/d) will normally be given a SCL of 0.01%.

As the estimated T25 value for DB[a,l]P is lower than 0.1 mg/kg bw/d an SCL of 0.001 % is proposed.

4.10.6 Conclusions on classification and labelling

Due to the clear evidence of carcinogenic activity in experimental animals DB[a,l]P should be classified and labelled as Category 1B carcinogen.

According to CLP Regulation DB[a,l]P has to be classified as:

Carc. 1B; and labelled with hazard statement H350: May cause cancer, with the pictogram "GHS08: Health hazard", and with the signal word "Danger".

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No data are available from humans exposed to dibenzo[def,p]chrysene alone. There are a number of epidemiological studies that show increased incidences of cancer in humans exposed to mixtures of PAH.

The carcinogenic potential of dibenzo[def,p]chrysene has been studied in numerous studies in three species under different protocols. Although no standard carcinogenicity study is available, the DS considered that the consistency of carcinogenic action from a broad series of studies are sufficient evidence of its carcinogenicity.

Repeated oral application of low doses of dibenzo[def,p]chrysene to two mouse strains induced benign and malignant tumours in various organs, e.g. ovaries, uterus, lymphoid tissues and skin; and oral squamous cell carcinoma after repeated applications of low doses on the tongue of hamsters.

Repeated dermal application of dibenzo[def,p]chrysene induced benign and malignant skin tumours (squamous cell papilloma and carcinoma) at high incidences in male and female mice. A dose-response relationship for tumour induction was observed in these studies. At low doses, tumours were observed to progress rapidly in size and killed the host within 46 weeks. In studies with higher doses, shorter latency periods were observed for tumour development in the skin of mice.

Following subcutaneous administration of dibenzo[def,p]chrysene (3 doses) to mice, sarcoma was observed at the site of administration.

Single i.p. injection dibenzo[def,p]chrysene to adult male mice induced lung adenomas in a dose-dependent manner. I.p. injections to newborn mice caused high incidences of be-

nign and malignant lung tumours to develop in both sexes. Dibenzo[def,p]chrysene treatment also induced high incidences of benign and malignant liver tumours and tumours in other organs.

Single intra-mammary injection of dibenzo[def,p]chrysene to rats caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in all treated animals and squamous cell carcinoma in the skin of 89 % of female rats.

In addition, dibenzo[def,p]chrysene was tested for tumour initiating potential in nine initiation–promotion studies on mouse skin. All nine studies reported positive responses by the increased frequency of skin papilloma when compared to negative controls.

The DS concluded that there was clear evidence of dibenzo[def,p]chrysene carcinogenicity in experimental animals. Together with the clear structural and mechanistic similarity of this substance to the established carcinogens chrysene and B[a]P, this supports a harmonised classification in Category 1B for carcinogenicity (H350).

As tumour development was noted following dermal administration of very small doses of dibenzo[def,p]chrysene, the T_{25} approach was used to calculate a specific concentration limit (SCL). The DS calculated a T_{25} value of 0.0005 mg/kg bw/day from the data provided in a carcinogenicity study that involved repeated topical application to the skin of female Swiss mice (Higginbotham *et al* (1993)). In accordance with the Guidance to Annex I of the CLP Regulation, this value corresponds to a high potency carcinogen (T_{25} ≤1 mg/kg/day).

Category 1B carcinogens showing high potency will normally be given a specific concentration limit an order of magnitude lower (0.01%) than the generic concentration limit (0.1%) for an ingredient to trigger classification of a mixture as a carcinogen. However, as the estimated T_{25} value for dibenzo[def,p]chrysene was considerably lower than 0.1 mg/kg, an SCL of 0.001% was proposed.

Comments received during public consultation

One Member State communicated their support for the proposal for classification as Carc. 1B and for the setting an SCL of 0.001%.

Assessment and comparison with the classification criteria

There are no standard, regulatory studies to inform on the carcinogenicity of dibenzo[def,p]chrysene, but there were two studies in mice and hamsters that involved administration by the oral route and five studies in the mouse that involved single or repeated dermal application. Other less conventional studies included i.p. or intra-mammary injection and sub-cutaneous administration to mice.

Oral studies

In a repeated dose carcinogenicity study, groups of female wild type mice (mixed genetic background of strains C57B1/6 and 129/Sv) received a dose of 0 or 1.07 mg/kg bw/day dibenzo[def,p]chrysene by gavage 5 times/week for 3 weeks. The study duration was 12 months, after which time dibenzo[def,p]chrysene was found to induce tumours in various organs (ovaries, lymphoid tissues and skin), as shown below.

The study also included an additional group of P450 1B1-null knock-out females.

Table: *Tumour findings in Wild Type and CYP1B1-knock-out mice following treatment with dibenzo[def,p]chrysene*

Finding	**Controls**	**Dibenzo[def,p]chrysene-treated groups**	
	Wild type	**Wild type**	**P450 1B1-null**
Survival rate	Not available	11/18 (61 %)	12/13 (92 %)
Total number of mice with tumours (benign + malignant)	4/27 (15 %)	17/17 (100 %)	8/13 (62%) (benign only)
Lung (adenoma)	1/27 (4 %)	0/17	5/13 (38 %)
Ovary (benign and malignant)	0/27	12/17 (72 %)	-
Skin (papilloma)	0/27	8/17 (47 %)	-
Lymphoma (malignant)	0/27	5/17 (29 %)	-
Lymphoma (follicular)	1/27 (4 %)	0/17	1/13 (8 %)
Uterus (Endometrial cystic hyperplasia)	1/27 (4 %)	0/17	5/13 (38 %)
Uterus (benign and malignant)	0/27	5/17 (29 %)	-
Liver (adenoma)	1/27 (4 %)	1/17 (6 %)	-
Liver (haemangioma)	-	-	1/13 (8 %)

In a second study, female Golden Syrian hamsters, dibenzo[def,p]chrysene was painted on to the tongue of females Golden Syrian hamsters, 5 times/week for 30 weeks (7 animals/group). Histopathology was performed on satellite groups of 3 animals after week 1, 6, 10 and 25.

After 1 week, small discrete areas of heterochromatic cells, hyperplasia, anaplasia and growth into connective tissue of the tongue mucosa were observed. After 6 weeks, moderate to severe dysplastic changes including pleomorphism, anaplasia, hyperplasia, hyperchromatism, mitotic figures and hyperkeratosis were observed. After 10 weeks these dysplastic changes were found to be severe and extensive proliferation into the underlying connective tissue was observed. After week 25, the development of oral squamous cell carcinoma was seen in 5/7 (71 %) hamsters (a total of 15 tumours). At the end of the study, this number had increased to 6/7 (85 %) hamsters (total of 18 tumours). No tumours were observed in untreated hamsters.

The results of these two oral studies, carried out in mice and hamsters, provide strong evidence of a carcinogenic effect following treatment with dibenzo[def,p]chrysene. The finding in the knockout mice serves to illustrate the importance of metabolic activation in the carcinogenic process.

Dermal studies

Five dermal studies are available, 4 of which were carried out by repeated dosing and 1 involved just a single application of dibenzo[def,p]chrysene. The latter study did not employ a concurrent control and is therefore of limited value.

In a study from 1966, Swiss Albino mice (20 females/group) were administered a topical application of dibenzo[def,p]chrysene (0, 0.86 mg/kg bw/day or 1.71 mg/kg bw/day in p-dioxan) 3 times/week for 12 months. After 15 months the study was terminated and the mice were assessed for skin tumours. In the low dose group, squamous cell papilloma and squamous cell carcinoma were both found in 17/20 (85 %) of the mice. The mean latency period was 245 days. In the high dose group, the incidence of both tumour types was

slightly higher: 18/20 (90 %). The mean latency period in this group was 210 days. No skin tumours were observed in the vehicle control group.

In a study published in 1972, Swiss Albino mice (19-21 females/group) received a topical dose of dibenzo[def,p]chrysene of 0, 0.017, 0.086, 0.17, 0.86 or 1.71 mg/kg bw/day, 3 times/week for up to 7 months:

***Table**: The number of doses each animal received in each dosing group*

Dose (mg/kg bw/day):	0	0.017	0.086	0.17	0.86	1.71
No. of doses:	55	55	40	24	7	7

B[a]P (0.05 % and 0.1 %) was used as a positive control in this study.

High incidences of skin tumours were observed in the treated mice, with a latency period as short as 56 days (0.86 mg/kg bw/day). Key findings are summarised in the following table.

***Table**: Number of mice with skin tumours following repeated dermal exposure of dibenzo[def,p]chrysene for up to 7 months (incidences shown by month)*

	Dibenzo[def,p]chrysene (mg/kg)					**B[a]P**	
	0.017	**0.086**	**0.17**	**0.86**	**1.71**	**0.05 %**	**0.1 %**
Total number of animals at start of study	20	19	21	20	21	20	20
	Number of animals with tumours/ number of surviving animals						
2 months	1/20	7/19	9/21	12/20	7/19	-	-
6 months	20/18	19/11	20/0	19/0	16/0	-	-
7 months	20/9	19/7	-	-	-	19/0	16/1
Mean latency	93 days	62 days	66 days	56 days	77 days	130 days	161 days

In a 1993 study (Higginbotham *et al*), dibenzo[def,p]chrysene was applied topically in acetone to the skin of female Swiss mice (22-27/group) at doses of 0, 0.3, 1.2 or 2.4 µg/day, 2 times/week for 40 weeks. High incidences of squamous cell carcinoma and/or papilloma were observed at the study close after 48 weeks. At doses of 1.2 µg/day and above, metastases were noted in the lungs and lymph nodes.

***Table**: Tumour incidence in the skin of mice treated with dibenzo[def,p]chrysene*

	Dose (µg/day)			
	0	**0.3**	**1.2**	**2.4**
No. mice with skin tumours/total no. in group	0/27	1/24 (4 %)	19/23 (83 %)	20/22 (91 %)
Squamous cell papilloma	-	1/24 (4 %)	9/23 (39 %)	16/22 (73 %)
Squamous cell carcinoma	-	-	16/23 (70%)	20/22 (91 %)
Mean latency	-	33 wks.	28±9 wks.	22±9 wks
Survival	-	47±2 wks.	46±3 wks.	43±8 wks.
Metastasis	-	-	Lung and lymph nodes (benign and malignant)	Lung and lymph nodes (benign and malignant)

Data from this study were used by the DS to derive a T_{25} value and to support their proposal for the setting of a specific concentration limit (see below).

In a fourth repeated dose dermal study (from 2004), groups of female wild-type C57BL/6J mice (17/group) and those with an aryl hydrocarbon receptor (AhR)-deficiency (AhR -/-) (15 /group) were treated first with a single dose of 1.2 mg/kg bw dibenzo[def,p]chrysene, followed by repeated doses of 34 µg/kg bw once/week for up to 20 weeks. For the wild type AhR (+/+) mice, the study duration was 24 weeks. For the AhR (-/-) mice, observations were extended for up to 2 years.

Dibenzo[def,p]chrysene induced a high incidence of skin tumours in AhR (+/+) mice following repeated dermal dosing. In AhR-deficient mice (AhR -/-), tumour development was less marked. In AhR (+/+) mice, all animals were found to have tumours by week 24. The first skin tumour appeared at week 11 and the average number of tumours per treated mouse was 2.7 ± 1.4. The tumours observed were squamous cell papilloma (76 %) and squamous cell carcinoma (24 %). In AhR (-/-) mice, 5/15 (33 %) were observed to have skin tumours by week 24. The first skin tumour was observed at week 21 and the average number of tumours per mouse was 0.46 ± 0.83. Only squamous cell papillomas were observed, no malignant tumours were seen. There were no further increases in tumour development on the skin during the follow-up period of up to 2 years. No data were available on mice treated with the vehicle control.

A study that investigated tumour formation after a single application of dibenzo[def,p]chrysene to the skin is also available. SENCAR[2] mice (24 females/group) were treated with a single dose of 30 µg of ± dibenzo[def,p]chrysene and then observed for a period of 27 weeks before sacrifice. At the end of the study, 7/24 mice were found to have skin tumours (4/7 squamous cell papilloma and 3/7 squamous cell carcinoma). It is difficult to reach any firm conclusions about the carcinogenicity of dibenzo[def,p]chrysene from this study given the absence of a negative control group, but the results are consistent with those of the other studies in mice.

Overall, these results provide a strong indication of the carcinogenic potential of dibenzo[def,p]chrysene following dermal application.

In addition, 9 studies of skin tumour initiation-promotion were included in the CLH report. They appear to have been well conducted, with the inclusion of appropriate controls, and all 9 gave clear positive results for tumour formation regardless of single or multiple administrations of dibenzo[def,p]chrysene as the initiator compound. These findings are typical of a carcinogen acting via a genotoxic mode of action.

<u>Other routes of exposure</u>

There were several other studies of carcinogenicity in laboratory animals included in the CLH report. Administration of dibenzo[def,p]chrysene to mice by sub-cutaneous, i.p. and intra-mammary injection has been found to increase the incidence of tumours at the application site. The results are consistent with the findings from the oral and dermal studies. However, given the non-physiological exposure routes used, these studies provide insufficient evidence for human hazard assessment and carcinogenicity classification and are not considered further in this opinion.

Comparison with the criteria

Conclusion and classification

Although no standard carcinogenicity studies are available with dibenzo[def,p]chrysene, there is consistent evidence of treatment-related tumour formation from both oral and dermal studies in mice and hamsters. Furthermore, action of this substance as an initiator in mouse skin tumour-initiation-promotion studies confirms the relevance for carcinogenicity of the positive genotoxicity studies described under Germ cell mutagenicity (above).

As there is no evidence available of carcinogenicity in humans being associated specifically with dibenzo[def,p]chrysene exposure, category 1A is not appropriate.

The observation of tumours in 2 species following treatment with dibenzo[def,p]chrysene, by 2 different routes of exposure, supported by a mechanism of action relevant to humans lead to the conclusion that a category 1B classification is justified for this substance, as shown in the following table.

Factor	Evidence with dibenzo[def,p]chrysene	Conclusion
Tumour type and background control	Mouse tumours in multiple organs, exceeding those in concurrent controls, following oral administration. In other studies, local tumours were formed at or near the site of administration and incidence exceeded concurrent controls (when included in the study).	Tumour types are relevant to humans - Cat 1B
Multi-site responses	Remote and local tumours were produced at different sites following multiple routes of exposure	Tumours formed at the expected sites of exposure in humans - Cat 1B
Progression of lesions to malignancy	Malignant tumours (uterus, ovary, lung, malignant lymphoma, squamous cell carcinoma) were reported in mice and/or hamsters.	Evidence of malignancy is sufficient for Cat 1B
Reduced tumour latency	Latency periods were short compared to total study durations.	This factor is indicative of potency but does not allow for differentiation between classification categories.
Whether responses are in single sex or both	Tumours increased in male and female animals.	Carcinogenic to both sexes - Cat 1B
Whether responses are in a single species or several	Tumour formation occurred in hamsters and mice	No evidence of a species specific response so it is likely relevant to humans - Cat 1B
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	Structural and mechanistic similarity to B[a]P and chrysene which are classified as Category 1B carcinogens	Cat 1B
Routes of exposure	Physiological (oral and dermal) and non-physiological routes of exposure	Oral and dermal study results confirm activity after exposure by a

	(sub-cutaneous, mammary gland, intra-peritoneal) produced tumours	physiological route - Cat 1B
Comparison of ADME between test animals and humans	No specific studies available, although it is well established that both animals and humans possess the metabolic capacity to activate PAHs through oxidative pathways.	Human relevance – Cat 1B
The possibility of a confounding effect of excessive toxicity at test doses	Tumours were not cited as a consequence of toxicity or other confounding factors by the DS	Tumours were a consequence of test substance exposure - Cat 1B
Mode of action and its relevance for humans	Postulated to be metabolised to reactive species with mutagenic activity	Mechanism is relevant to humans - Cat 1B

Based on the available data and comparison to the criteria of CLP (see above), RAC is in agreement with the DS that classification of dibenzo[def,p]chrysene for **carcinogenicity in Category 1B is appropriate**.

Calculation of SCL

The DS assessed the carcinogenic potency of dibenzo[def,p]chrysene using standard methodology, deriving an oral T_{25} value of 0.00051 mg/kg bw/day based on the skin tumour frequency seen in female mice following dermal administration (study by Higginbotham *et al*. 1993).

As mentioned by the DS, the T_{25} method for calculation of potency relies conventionally on data from studies that fulfil a number of criteria:

a) the test animals should be mammals,
b) administration of the test substance should begin early in life (preferable from time of weaning, but up to 100 days is acceptable for rats, mice and hamsters),
c) the route of administration should be via the diet, drinking water, by gavage or inhalation,
d) the test substance should be bioavailable for systemic absorption,
e) the test agent was administered alone,
f) exposure was chronic, with no more than 7 days between each dose
g) the duration of exposure was at least one-fourth of the standard study period for that species,
h) the duration of experiment was at least half of the standard lifespan for that species,
i) the study design has included a control group,
j) the study design included at least 10 animals per group,
k) the pathology data were reported for the number of animals with tumours rather than total number of tumours, and
l) the results reported were original data.

In the absence of such a complete set of information, data from experiments fulfilling as many as possible of these conditions are preferred. The assumptions made by using experiments not fulfilling regulatory guidelines should be specified and justified by toxicological considerations. Accordingly, the DS acknowledged that the study used to calculate T_{25} was a dermal study with a duration of less than ¼ of that of a standard carcinogenicity study and therefore did not strictly meet the above criteria. However, the DS made the

assumption that absorption of dibenzo[def,p] chrysene would occur for all routes and considered that the sensitivity of various tissues in carcinogenicity studies involving the oral route of exposure supported the use of this approach for the estimation of potency.

This justification, together with the general observation that high incidences of tumours can be induced in animals following relatively short treatment periods involving relatively low doses (considerably less than 10 mg/kg/day over a conventional 2-year period), shows clearly that it can be regarded as a high potency carcinogen.

According to CLP, high potency carcinogens (T_{25} < 1 mg/kg bw/day) are assigned an SCL of 0.01 %. However, given that the calculated T_{25} was more than 10-fold less than the cut-off value, the DS proposed to assign an SCL of 0.001 %.

In principle, RAC agrees with this proposal. All 3 substances would appear to be high potency carcinogens as defined in the CLP Regulation – see, for example, the comparative data in the following table.

***Table**: Comparative potencies of 3 PAHs causing skin tumours in female Swiss mice following dermal treatment*

Substance	Dosing regimen	Tumour Type indicated
Dibenzo[b,def]chrysene	1.13 mg/kg bw/day 2x/week for 70 weeks	89.7 % mice with tumours - 45 % increase in squamous cell carcinoma, - 17 % increase in skin papilloma
Benzo[rst]pentaphene	~ 0.86 mg/kg bw/day 3x/week for 52 weeks	80 % increase in skin papilloma, 65 % increase in skin epitheliomas
Dibenzo[def,p]chrysene	1.2 µg/day (~0.05 mg/kg bw/day) 2x/week for 40 weeks	83 % mice with tumours - 70 % increase in squamous cell carcinoma - 39 % increase in skin papilloma

After comparing the relative potencies of the 3 PAH substances in female Swiss mice following dermal application for up to 70 weeks, it is clear that all 3 substances cause cancer at very low doses (0.05 - 1.13 mg/kw bw/day). However, the dose at which dibenzo[def,p]chrysene causes skin tumours in these studies is about 20 times lower than the other PAHs (approximately 0.05 mg/kg bw/day), and therefore on this basis it could be considered the most potent of the three.

In the recently drafted RAC opinion documents for dibenzo[b,def]chrysene and benzo[rst]pentaphene, SCLs were not proposed for this endpoint. No justification was provided by the DS as to why a SCL should not be considered for these substances. Nevertheless, given the very high potency of dibenzo[def,p]chrysene that has been seen in animals, RAC agrees with the proposal to set a SCL for this substance. RAC agrees with the DS that **an SCL of 0.001%**, which is 10-fold lower than the limit routinely set for high potency carcinogens, **is warranted**. This accounts for the exceptionally low concentrations and short exposure periods needed to produce tumours in these studies.

4.11 Toxicity for reproduction

No data are available for this toxicological endpoint.

4.12 Other effects

No data are available for this toxicological endpoint.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Hazard classes not assessed in this dossier.

6 OTHER INFORMATION

7 REFERENCES

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8 ANNEXES

Annex I

Setting specific concentration limits (SCL) for carcinogens: Determination of the carcinogenic potency of DB[a,l]P

Experimental studies have revealed large variations in the doses of various carcinogenic substances needed to induce tumours in animals. Thus, the amounts of chemical carcinogens required to induce tumours vary with a factor of up to 10^8-10^9 for different compounds. It is reasonable to assume that there is similar variation in the potency of substances carcinogenic to humans (Sanner and Dybing 2005).

CLP, Article 10.1 allows the use of SCLs based on the potency of the carcinogen(s). The EU has adopted the T25 concept for carcinogenicity (Dybing et al. 1997) with additional considerations as a measure for intrinsic potency and a guidance document (EC 1999) to assist in establishing SCLs for carcinogens. By using this approach the SCL may occasionally be reduced or raised from the default generic concentration limits.

Determination of the T25 value

T25 was expressed in 'mg/kg bw/d'. This value is used to allocate carcinogenic substances to the high, medium or low potency class. The potency class and the CLP category for carcinogens finally define the concentration limits of the substances in a mixture.

The T25 is calculated from the data that are also used in the CLH dossier for DB[a,l]P. The available data for carcinogenicity of DB[a,l]P give clear evidence of carcinogenic activity in experimental animals. DB[a,l]P fulfils the criteria for classification and labelling as Category 1B carcinogen, H350 (May cause cancer) according to CLP.

Selection of the most suitable study for calculation of T25

There is no carcinogenicity study with full compliance to the present OECD/EU test guidelines. The majority of long-term studies used the dermal route. The only available gavage study is limited to a 3 week treatment period followed by an observation period without treatment of up to 12 months (Buters et al. 2002). Therefore a dermal study in mice (Higginbotham et al. 1993) with three doses, showing dose-dependent high tumour rates, which examined the skin and a number of other organs and diagnosed differentiation into benign and malignant tumour types, was selected for T25 calculation.

→ Calculation of T25 for developing skin tumours (squamous cell carcinoma and papilloma) at high incidences after repeated dermal application of low doses to female Swiss mice (Higginbotham et al. 1993) (for study details and results see Section 4.10.1.3 'Carcinogenicity; dermal'; Table 20)

The data of skin tumour development in female mice were assessed as suitable for the T25 approach, although this study does not follow the EU/OECD standard test guidelines for carcinogenicity using standard routes and daily dosing.

Skin tumours (squamous cell carcinoma and papilloma) occurred in mice at all of the three tested dose groups (1, 4 or 8 nmol, approx. 0.3, 1.2 or 2.4 µg/d; application 2x/week (painting on shaved dorsal mouse skin) for up to 40 weeks) at the incidences of 1/24, 19/23, and 20/22, respectively. In the control group none (0/27) was found. The lowest dose with a significantly increased incidence of skin tumours was used for calculation. This concentration was 4 nmol (approx. 1.2 µg/d).

Adjustment of background tumour incidences is needed (Dybing et al., 1997)

Skin tumours: 4 nmol (approx. 1.2 µg) DB[a,l]P in 100 µL acetone was tested by repeated application (painting on shaved dorsal mouse skin) twice weekly for up to 40 weeks; default value for body weight mouse: 0.03 kg

1.2 µg (calculated ≈ 40 µg/kg bw): 19/23 (83 %, $p<0.001$), 16/23 squamous cell carcinoma (70 %) 9/23 squamous cell papilloma (39 %); Control: 0/27; net% = 83 %

Modification of the starting point: Conversion of the mouse LOAEL into a corrected human LOAEL:

Correction of the exposure conditions from the mouse study into human exposure conditions:

Lifetime risks for consumer and for humans exposed indirectly via the environment are associated with daily exposure for 24 hours (7 days a week). Therefore correction is needed for

- 2 days/week to default consumer 7 days per week:

$LOAEL_{corr}$ = (2/7) * 40 µg/kg bw = 11.43 µg/kg bw = 0.01143 mg/kg bw

If dosing is terminated at w weeks (w < the standard lifespan of 104 weeks), the dose giving a tumour in 83 % of the animals is corrected by w/104:

- 40 weeks to default the standard lifespan of 104 weeks:

$LOAEL_{corr} = (40/104)^2 * 0.01143$ mg/kg bw = 0.001690828402 mg/kg bw/d

The chronic dose rate giving skin tumours in 25 % of the animals (T25) for DB[a,l]P is calculated from 0.001691 mg/kg bw/d : T25

= (25/83) * 0.001691 mg/kg bw/d = 0.000509337349 mg/kg bw/d

The relevant dose descriptor is T25 = 0.0005 mg/kg bw/d (based on results from a non-guideline carcinogenicity study by repeated topical application to the skin of female mice by Higginbotham et al. 1993).

Based on the experimental data of the study by Higginbotham et al. (1993) a **T25 value of 0.0005 mg/kg bw/d for DB[a,l]P** is estimated in mice.

For comparison with the T25 value after dermal application in mice also a T25 value was derived with data from an oral cancer model (a non-guideline carcinogenicity study by painting on the tongue) to female Golden Syrian hamsters (Schwartz et al. 2004; for study details and results see Section 4.10.1.1 ‘Carcinogenicity; oral’; Table 19). Development of squamous cell carcinoma around the oral cavity occurred after repeated mucosal application of 3 µg, 5x/week, for a period of 30 weeks, in 86 % of the animals. In the control group none was found. Calculation of T25 for developing oral squamous cell carcinoma after repeated mucosal application of 3 µg (5x/week for 30 weeks) on the tongue of hamsters (corrected for hamster body weight, 3 µg/0.11 kg bw hamster; for a complete week, 5/7 days; for standard lifespan exposure, 30/104 weeks; and tumour rate of 25 %, 25/86) resulted in a **T25 value of 0.00047 mg/kg bw/d for DB[a,l]P** for hamster in this oral long-term study.

For the purpose of assigning specific concentration limits, it is proposed that:

* Carcinogens of high potency: T25 value ≤ 1 mg/kg bw/d.

Note on the dermal route

In the 'Guidelines for setting specific concentration limits for carcinogens' for setting specific concentration limits for carcinogens in Annex I of Directive 67/548/EEC inclusion of potency considerations' (EC 1999) there is a reference using local effects in the T25 derivation. On page 13 authors stated: " For studies involving i.e. a non-systemic contact carcinogens it is difficult to calculate the T25 value, the potency grading should be made on a case by case basis." Assuming that absorption of DB[a,l]P will occur for all routes (dermal, oral, inhalation) and taking into account that oral administration caused tumours at other sites (see Buters et al. 2002), the dermal studies are in general considered as relevant. Even after correction for the absorption rates of 50 % or 20 % following dermal application derived in the 'Annex XV restriction report for the 8 PAH (2010)', p. 50 (see there)[3], the resulting T25 value would be far below 0.1 mg/kg bw/d.

Conclusion: Specific concentration limits for DB[a,l]P, Category 1B carcinogen

According to the 'Guidelines for setting specific concentration limits for carcinogens' for setting specific concentration limits for carcinogens in Annex I of Directive 67/548/EEC inclusion of potency considerations' (EC 1999) and the 'Guidance on Application of the CLP Criteria' (Version 4.1, June 2015, Section 3.6.2.5) DB[a,l]P belongs to the carcinogens of high potency (T25 value $\leq$ 1 mg/kg bw/d).

On the basis of the guidance (EC 1999) described in Chapters 3 and 4a potency evaluation has been made. From this potency evaluation SCL for carcinogens can be derived.

Category 1B carcinogens showing high potency will normally be given a specific concentration limit an order of magnitude lower (0.01 %) than the generic concentration limits of ingredients of a mixture classified as carcinogen that trigger classification of the mixture of $\geq$ 0.1% (see EC 1999). In accordance with these guidance Category 1B carcinogens showing high potency will normally be given a SCL of 0.01%.

As the estimated T25 values for DB[a,l]P are < 0.1 mg/kg bw/d an SCL of 0.001 % is proposed.

[3] http://www.bfr.bund.de/cm/343/pak_annex_XV_restriction_report_proposal_for_a_restriction.pdf;

http://echa.europa.eu/addressing-chemicals-of-concern/restrictions/substances-restricted-under-reach/-/dislist/details/0b0236e1807e2cba

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