CLH report

Proposal for Harmonised Classification and Labelling

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

dibenzo[b,def]chrysene

EC Number: **205-878-0**

CAS Number: **189-64-0**

Index Number: **n.a.**

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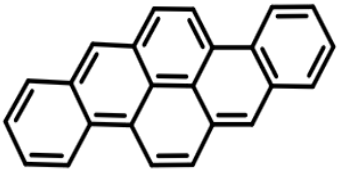
TABLES

1. IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	dibenzo[b,def]chrysene
Other names (usual name, trade name, abbreviation)	Dibenzo[a, h]pyrene Dibenzo[*b,def*]chrysene 3,4:8, 9-Dibenzopyrene
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	205-878-0
EC name (if available and appropriate)	Dibenzo[*b,def*]chrysene
CAS number (if available)	189-64-0
Other identity code (if available)	-
Molecular formula	$C_{24}H_{14}$
Structural formula	
SMILES notation (if available)	C1=C4C3=C2C(=C1)C6=C(C=C2C=CC3=C5C(=C4)C=CC=C5)C=CC=C6
Molecular weight or molecular weight range	302.37 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	≥80 % w/w

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
dibenzo[b,def]chrysene			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No impurity is considered relevant for the classification of the substance dibenzo(a,h)pyrene.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additive is considered relevant for the classification of the substance dibenzo(a,h)pyrene.					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposal of classification and labelling

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-			
Dossier submitters proposal	tbd	dibenzo[b,def]chrysene	205-878-0	189-64-0	Muta. 2 Carc. 1B	H341 H350	GHS08 Dgr	H341 H350			
Resulting Annex VI entry if agreed by RAC and COM	tbd	dibenzo[b,def]chrysene	205-878-0	189-64-0	Muta. 2 Carc. 1B	H341 H350	GHS08 Dgr	H341 H350			

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/ irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	data lacking	No
Skin sensitisation	data lacking	No
Germ cell mutagenicity	harmonised classification proposed	Yes
Carcinogenicity	harmonised classification proposed	Yes
Reproductive toxicity	data lacking	No
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No

Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

This CLH proposal aims to classify and label of dibenzo[b,def]chrysene (dibenzo(a,h)pyrene; DB[a,h])P) for mutagenicity and carcinogenicity. DB[a,h]P is not listed in Annex VI to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Dangerous Substances (CLP Regulation).

So far, DB[a,h]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 for Classification and Labelling (TC C&L) (Dir. 67/548/EEC).

DB[a,h]P is a 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 Regulation up to and including 2013 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), benzo[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 dibenzo[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.

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

Based on the results both of in vitro testing (bacteria; proliferating cells of cell cultures) and in vivo testing (soma cells) DB[a,h]P is evaluated as genotoxic by international bodies (SCF 2002; FAO/WHO 2006; EFSA 2008; IARC 2010) and by an international regulation program (IPCS 1998). Since these evaluations, no further data has become available. For the justification of classification as mutagen according to CLP Regulation a re-evaluation of the available mutagenicity/genotoxicity has been carried out.

The carcinogenic potential of DB[a,h]P was first evaluated in December 1972 by a Working Group of IARC. Bioassays in mice revealed squamous cell papilloma and epithelioma (the Working Group considered these tumours to be squamous cell carcinoma) in the skin after repeated dermal application. Sarcomas were induced after subcutaneous injections in mice (IARC 1973).

In February 1983 a Working Group of IARC re-assessed the same bioassays and concluded that there was sufficient evidence that DB[a,h]P was carcinogenic to experimental animals (IARC 1983). On the basis of these data the working group classified DB[a,h]P according to their classification system as possible carcinogenic to human beings (group 2B).

In a subsequent evaluation by IARC in 2006 additional bioassays were included. These were a study on mouse skin, three dermal initiation–promotion studies in mice, a study of intra-peritoneal administration to newborn mice, and a further study of administration in the mammary gland of rats. All studies gave positive results. DB[a,h]P was confirmed as possible carcinogenic to human beings (group 2B), on the basis of sufficient evidence in animals (Straif et al. 2005; IARC 2006).

The same experimental studies on DB[a,h]P are now re-evaluated for classification according to CLP Regulation. Following studies that were not taken into account in the earlier IARC monographs are considered additionally: a study by subcutaneous implantation of a paraffin disk containing DB[a,h]P in rats, and a further dermal initiation–promotion study in mice.

4. 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 Regulation.

5. 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,h]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.

6. DATA SOURCES

The evaluation of the toxicity of PAH was reviewed in a variety of international bodies/regulatory programs (ATSDR (1995), IARC (1983, 2010), IPCS (1998), EFSA (2008), SCF (2002), US EPA (1984) and WHO (1998, 2003)). Some reports have also assessed the toxicological data on DB[a,h]P.

In addition to the above mentioned review reports, which covered the published literature up to and including 2005, a literature research including publications on DB[a,h]P was performed for this dossier in order to account for the recent literature on DB[a,h]P and PAH toxicity from 2006 until June 2014.

Literature was searched in the online databases DIMDI (medline, xtoxline), Scopus, Chem ID plus Advanced (pubmed, ToxNet), ISI Web of Knowledge and Chemical Abstracts starting from 2006. The databases were queried for the terms ‘PAH’ and the substance name ‘dibenzo(a,h)pyrene’ and the CAS Number in combination with the terms ‘muta’, ’cancer’ or ‘carc’, ‘tumour’ or ‘tumor’, ‘sensitisation’, ‘repeated’, and ‘repro’.

No registration dossiers are available (June 2016).

7. PHYSICOCHEMICAL 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,h]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 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Golden-yellow plates, recrystallized from xylene or trichlorobenzene.	Lide, D.R., ed. (**2005**), *CRC Handbook of Chemistry and Physics*, 86th Ed., Boca Raton, FL, CRC Press.	
Melting/freezing point	315 °C	Lide, D.R., ed. (**2005**), *CRC Handbook of Chemistry and Physics*, 86th Ed., Boca Raton, FL, CRC Press.	
Boiling point	596°C	Greim H. (Hrsg), (**2008**), *Polycyclische Aromatische Kohlenwasserstoffe. Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten*, 45. Lieferung, Wiley-VCH, Weinheim	
Relative density	n.a.		
Vapour pressure	$6.41*10^{-12}$ mm Hg at 25 °C	SRC PhysProp Database, 2012 (secondary source)	estimated
Surface tension	n.a.		
Water solubility	$3.5*10^{-5}$ mg/L at 25 °C	SRC PhysProp Database, 2012 (secondary source)	estimated
Partition coefficient n-octanol/water	log P_{OW} 7.28 at 25 °C	SRC PhysProp Database, 2012 (secondary source)	estimated
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.		

8. EVALUATION OF PHYSICAL HAZARDS

Hazard classes not assessed in this dossier.

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of a toxicokinetic study relevant for carcinogenicity

Method	Results	Remarks	Reference
Metabolism in vitro HPLC of the metabolites formed in vitro from DB[a,h]P	**Formation of metabolite** → highly reactive compounds DB[a,h]P was metabolized to two dihydrodiols: a 'bay-region' diol, trans-1,2-dihydroxy-1,2-dihydrodibenzo[a,h]pyrene (dibenzo[a,h]pyrene-1,2-diol), and trans-3,4-dihydroxy-3,4-dihydrodibenzo[a,h]pyrene by an Aroclor 1254-induced rat liver preparation	Evidence of metabolism and metabolic activation of DB[a,h]P	Hecht et al. 1981

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The general principles of the kinetics of PAH, in particular B[a]P, have been covered exhaustively in the published literature. Data of 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,h]P are not available a general overview of the toxicokinetics of PAH is given.

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.

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 fetal 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 P-450-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.

In a metabolism study (in vitro) it was shown that DB[a,h]P is metabolized to the proximate bay-region diol, dibenzo[a,h]pyrene-1,2-diol, and trans-3,4-dihydroxy-3,4-dihydrodibenzo[a,h]pyrene by rat liver preparations.

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.

10. EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

For the toxicological endpoint respiratory sensitisation no data are available.

10.7 Skin sensitisation

Hazard class not assessed in this dossier.

10.8 Germ cell mutagenicity

Table 10a: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Bacterial gene mutation test (Ames test) not in accordance with OECD TG 471 crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: not known	- S. typhimurium tester strains: TA 98 and TA 100 - tested only with metabolic activation - tested concentrations: 6.125 - 50 nmol	Result: **positive** effect: weakly positive in TA 98 in the entire dose range cytotoxicity: no effect deficiency: only graphical presentation without detailed information	Wood et al. 1981
Bacterial gene mutation test (forward mutation) no guideline available crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: > 96 %	- S. typhimurium tester strain: TM 677 - tested with and without metabolic activation - tested concentrations: up to 30 µg/mL	Result: **negative** cytotoxicity: no effect deficiency: - only graphical presentation - lack of detailed experimental data	Busby et al. 1995
DNA repair test (SOS chromotest) no guideline available deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: > 99 %	- E. coli tester strain: PQ37 - tested with and without metabolic activation - tested concentrations: 0.052 – 3.3 µg/assay	Result: **positive** effect: positive only with metabolic activation at 3.3 µg/assay cytotoxicity: no effect	Mersch-Sundermann et al. 1992

Gene mutation test (TK locus) compliant to OECD TG 476 GLP: no information	**DB[a,h]P** Purity: > 99.2 %	- cell type: h1A1c2 cells - tested only with metabolic activation (see cell type) - tested concentrations: 0.1, 1.0, 10.0 and 100.0 ng/mL - The cell line was derived from human B-lymphoblastoid cells - The cells have been engineered to express cytochrome P4501A1 (CYP1A1), an enzyme capable of metabolizing PAHs. - The testing was done at low doses to measure mutagenicity at concentrations reflecting the low levels of PAHs reported in environmental samples.	Result**: positive** effect: positive at concentrations of 10.0 and 100.0 ng/mL (MF: 2.4 and 4.2) cytotoxicity: 100.0 ng/mL induced toxic effects controls: relevant negative and positive control deficiency: mutagenicity was measured at TK locus, but there is no differentiation of colony size	Durant et al. 1999
Gene mutation test (TK locus) not in accordance with OECD TG 476 crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: > 96 %	- cell type: MC-5 cells - tested with metabolic activation (see cell type) - tested concentrations: 0.05 - 1.7 µg/mL - The cell line derived from human B-lymphoblastoid cells. - The cells contain activity for five cytochromes P450 and microsomal epoxide hydrolase.	Result: **negative** cytotoxicity: at the two highest tested concentrations deficiencies: - only graphical presentation - lack of detailed experimental data	Busby et al. 1995
Gene mutation tests (HPRT) compliant to OECD TG 476 GLP: no information	**DB[a,h]P** Purity: not known	- cell type: V79 cells - tested with and without metabolic activation (see: cell mediated assay) - tested concentrations: 0.03 - 0.3 µg/mL - Cell mediated assay: use of golden hamster embryo cells which are capable of metabolizing PAH	Result: **positive** effect: positive only with use of golden hamster embryo cells from 0.03 µg/mL upwards in a dose dependent manner cytotoxicity: from 0.1 µg/mL upwards controls: relevant negative and positive control	Hass et al. 1982

Table 10b: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Determination of DNA adducts (^{32}P-postlabeling analysis) no guideline available crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: > 99 %	Species: - Parkes mice - 4 males /group Target cells: - skin cells (cells of treated skin area) Exposure and harvest time: - application of 1.0 µmol DB[a,h]P/mouse in 200 µl tetrahydrofurane - topical treatment; shaved dorsal skin was used for application - animals were killed 7, 21, 84 days after treatment	Result: **positive** effect: - two adduct spots regardless of the solvents - maximum adduct formation of 3.0 fmol/µg DNA after 2 days toxicity: no information	Hughes and Phillips 1990
Determination of DNA adducts (^{32}P-postlabeling analysis) no guideline available crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: not known	Species: - Parkes mice - 4 males /group Target cells: - lung cells Exposure and harvest time: - application of 1.0 µmol DB[a,h]P/mouse in 200 µl tetrahydrofurane - topical treatment; shaved dorsal skin was used for application - animals were killed 7, 21, 48 days after treatment	Result: **positive** effect: two adduct spots regardless of the solvents toxicity: no information	Hughes and Phillips 1990

Determination of DNA adducts (^{32}P-postlabeling analysis and fluorescence line narrowing spectroscopy (FLNS)) no guideline available crucial deficiency: no positive control GLP: no information	**DB[a,h]P** Purity: not known	Species: - Parkes mice - 4 males /group Target cells: - skin cells (cells of treated area) Exposure and harvest time: - application of 1.0 µmol DB[a,h]P/mouse in 200 µl tetrahydrofurane - topical treatment; shaved dorsal skin was used for application - animals were killed 2 days after treatment	Result: **positive** effect: - two adducts were identified with each method - postlabeled adducts 1 and 2 are identical with the adducts I and II that were identified by the FLNS method - the profile of both adducts is essentially the same as described by Hughes and Phillips (1990)	Marsch et al. 1992

Table 10c: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No human data for germ cell mutagenicity are available.				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Positive genotoxicity tests both in vitro and in vivo are available. A re-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 (gene mutation test at TK locus; HPRT test) 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 test is to be considered for justification of classification of DB[a,h]P as mutagen, although the other positive results from in vitro/in vivo testing seem to be conclusive.

Experimental data *in vitro*

DB[a,h]P induces mutagenic effects in bacteria and in exposed proliferating cells of mammalian and human cell lines. These effects were only observed in the presence of an exogenous metabolic system.

DB[a,h]P induces gene mutation in S. typhimurium tester strain TA 98 (Wood et al. 1981) as well as DNA damage in E. coli tester strain PQ37 (Mersch-Sundermann et al. 1992).
A gene mutation test with the S. typhimurium tester strain TM 677 was negative with and without metabolic activation.

In mammalian cell gene mutation tests DB[a,h]P induces positive effects in V79 cells and in cells of a cell line derived from human B-lymphoblastoid cells (h1A1v2 cells).
DB[a,h]P induces gene mutations in V79 cells in a cell mediated assay (Hass et al. 1982). Since V79 cells do not metabolize PAH the induction of mutagenic effects was tested in the presence and absence of golden hamster embryo cells capable of metabolizing PAH. In the presence of hamster embryo fibroblasts DB[a,h]P was mutagenic in a dose-dependent manner in the tested range from 0.03 up to 0.3 µg/mL. Cytotoxic effects were observed from 0.1 µg/mL upwards. No mutagenic effect was observed in the absence of the hamster embryo cells.
In a TK-mutation test a positive effect was observed at the thymidine kinase (Tk) locus in h1A1v2 cells at the two highest tested concentrations of 10 and 100 ng/mL (Durant et al. 1999). The cells had been engineered to express cytochrome P4501A1 (CYP1A1), an enzyme capable of metabolizing PAH. Cytotoxic effects were observed at the highest tested concentration of 100 ng/mL. The positive result cannot be assessed in detail, because no differentiation into small colonies, suggestive of chromosomal aberrations, or large colonies, suggestive of gene mutations, was carried out. Because there is no colony sizing no conclusion can be drawn whether gene mutations or chromosomal aberrations were induced preferentially.
In a further TK-mutation test with MCL-5 cells DB[a,h]P induced no mutagenic effects up to the highest tested concentration of 1.7 µg/mL (Busby et al. 1995). Higher concentrations were characterised as excessively toxic.

Experimental data *in vivo*

DB[a,h]P induces genotoxic effects (DNA adducts) in soma cells.

DB[a,h]P induces DNA adducts in skin cells as well as in lung cells of mice after treatment with 1µmol applied onto the shaved dorsal skin (Hughes and Phillips 1990). Two different adducts were found in cells of the mouse skin. According to the authors these adducts were detected in much smaller quantities also in lung cells.
Under similar experimental conditions (e.g. application of 1 µmol DB[a,h]P/mouse; application onto the shaved dorsal skin) Marsch et al. (1992) identified also two adducts. According to the authors the profile of adducts was essentially the same as described by Hughes and Phillips (1990).

Validity of the test systems

Only two positive in vitro mutagenicity tests (gene mutation test at Tk locus; HPRT test) were carried out in accordance with an EU/OECD test guideline.

As described previously the data for mutagenicity of DB[a,h]P were obtained from in vitro (bacteria; proliferating cell cultures) and in vivo testing (soma cells). Based on the results of these tests DB[a,h]P was evaluated as genotoxic by international bodies (SCF 2002; FAO/WHO 2006; EFSA 2008; IARC 2010) and by an international regulation program (IPCS 1998).

A re-evaluation of the available mutagenicity/genotoxicity tests taking into account the quality of the test performances leads to the conclusion that only two positive in vitro mutagenicity tests (gene mutation test at Tk locus (Durant et al. 1999); HPRT test (Haas et al. 1982)) were carried out in accordance with an EU/OECD test guideline. The lack of positive controls in all other positive tests is a crucial methodological shortcoming. Therefore, 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 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. Therefore, only the guideline compliant positive in vitro gene mutation tests were considered for the justification of classification of DB[a,h]P as mutagen.

Read-across approach

A read-across approach between DB[a,h]P and B[a]P as well as CHR is justified.

B[a]P and a number of further PAH, e.g. DB[a,h]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, whereas CHR (CAS 218-01-9) is classified/labelled as Cat. 2, H341 according to CLP Regulation.

PAH with relatively planar, highly conjugated aromatic structures such as B[a]P and CHR have a genotoxic potential which is characterised as indirect because metabolic activation is required for the induction of genotoxic effects. Mutagenic effects are induced after biotransformation to reactive electrophilic metabolites (dihydrodiol epoxides), which bind covalently and form adducts with intracellular macromolecules such as DNA adducts. DNA adducts, which may result in gene mutations, DNA strand breaks or chromosomal aberrations, are the precursor lesions for mutations, which arise through replication of errors in the DNA during DNA synthesis.

B[a]P (five-ringed PAH) and CHR (four-ringed PAH), with at least one reactive so-called bay-region (structural element in the PAH due to the linkage of the ring systems), are metabolised mainly through the bay-region dihydriol epoxides pathway. This pathway is catalysed 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). The formation of diolepoxides that covalently bind to DNA appears to be the primary step in the mechanism of action of genotoxic unsubstituted PAH.

DB[a,h]P possesses two bay-regions and belongs to the group of relatively planar, highly conjugated six-ringed dibenzopyrenes. In a reliable study Hass et al. (1982) examined not only the induction of gene mutations in V79 cells but also sought to clarify the question whether the metabolic oxidation at the bay-regions is required for a mutagenic response. Since V79 cells do not metabolize PAH, mutagenesis was tested in both the presence and the absence of hamster embryo cells capable of metabolizing PAH (cell mediated assay). It was shown that DB[a,h]P was only mutagenic in the presence of hamster embryo cells. Due to this positive result the role of the bay-regions in the activation of DB[a,h]P has been investigated. First it was investigated whether metabolic oxidation of DB[a,h]P at the bay-regions is necessary for the mutagenic response. Therefore the involvement of microsomal oxidation in the metabolism of DB[a,h]P was tested by using 7,8-benzoflavone (BF) a known inhibitor of mixed-function oxidases. It could be shown that the induction of mutagenic effects was prevented by additional incubation of DB[a,h]P with BF. Furthermore it could be demonstrated that the difluorinated derivate of DB[a,h]P, 3,10-difluorodibenzo[a,h]pyrene, extremely inhibited the induction of mutagenic effects in V79 cells. This effect implies the involvement of the particular fluorinated carbon atoms at positions of the bay-regions in the metabolic activation of the parent compound. In summary, it can be concluded, that a metabolic oxidation

(presumably via oxidative pathway) at the bay-regions is required for the mutagenic response of DB[a,h]P in this assay.

Due to their chemical structures and the resulting metabolic activities at the bay-region(s) a read-across approach between DB[a,i]P, B[a]P and CHR is sufficiently justified. The read-across approach is based on the following common substance properties:

- The substances have planar, highly conjugated aromatic structures.
- The substances require metabolic activation for the induction of mutagenic/genotoxic effects.
- The bay-regions were identified as common reactive centre.
- Dihydrodiol epoxides are formed as common breakdown products via biological processes at the bay-region(s).
- The substances induce genotoxic effects *in vitro* and *in vivo* after metabolic activation at the bay-region.

Following the read-across approach it is justified to use the classification of B[a]P and CHR as mutagen as a supporting argument for justification of classification of DB[a,h]P as mutagen.

10.8.2 Comparison with the CLP 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,h]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,h]P. The available guideline-compliant in vitro gene mutation tests are not sufficient as justification for classification of DB[a,h]P as a Category 2 mutagen.

But the CLP Regulation (Annex I, point 3.5.2.2, p. 145) 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 requirements are fulfilled for DB[a,h]P.

In principle PAH have repeatedly shown to have genotoxic effects both in vivo in soma cells of rodents and in vitro in mammalian (inclusive human) cell lines and procaryotes. Due to the chemical structures and its resulting ability to induce mutagenic/genotoxic effects in vitro and in vivo a read-across approach between DB[a,h]P, B[a]P and CHR is considered of sufficient relevance. The read-across approach clearly supports the relevance of the positive mutagenic/genotoxic effects in vitro and in vivo in soma cells induced by DB[a,h]P despite the lack of positive controls in most of the positive mutagenicity tests. Therefore, it is also reasonable to conclude that the classification of B[a]P and CHR as mutagen can be used as supporting criterion for justification of classification of DB[a,h]P as a mutagen.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

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

10.9 Carcinogenicity

Table 11a: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of expo-sure	Results	Reference
Carcinogenicity study by topical application to the skin no guideline 9 synthesized and/or highly purified hexacyclic aromatic hydrocarbons were tested for carcinogenicity and tumour-initiating potency on mouse skin Dose group: 2 Control group: 1 Application: 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: 20 treated with the vehicle p-dioxane alone	**DB[a,h]P** purified by chromato-graphy followed by recry-stallization	0.05 or 0.1 % in p-dioxan 3x/wk, for 12 months	**Positive** **DB[a,h]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,h]P** 16/20 (**80 %**; squamous cell papilloma) 13/20 (**65 %**; squamous cell carcinoma) Mean latency period: 224 days **0.1% (≈1.71 mg/kg bw/d) DB[a,h]P** 15/20 (**75 %**; squamous cell papilloma) 15/20 (**75 %**; squamous cell carcinoma) Mean latency period: 196 days **Control:** no skin tumour, 0/20	Hoffmann and Wynder 1966 LaVoie et al. 1979

Carcinogenicity study by skin painting no guideline Dose group: 1 Dosage not specified Control group: none Application: 2x/wk until occurrence of tumours no statistics	**Mouse** strain and gender not specified 30/group	**DB[a,h]P** Purity: not specified, but described as pure	Dosage not specified Vehicle: Benzene (chiefly 0.3 % solution) 2x/wk until tumours occurred	**Positive** **DB[a,h]P induced squamous cell carcinoma** (epithelioma) Squamous cell carcinoma on the application site was found in **10/30 mice** (**33 %**). Two mice bearing epithelioma had also metastases in the lung. The earliest day of death of tumour-bearing mice was on day 202 and the latest day of death on day 350 of treatment.	Badger et al. 1940
Carcinogenicity study by topical application to the skin no guideline comparative study with other PAH Dose group: 1 Control group: 1 Application: 2x/wk (onan area of 1.5 cm^2), for a period of 30 wk, thereafter observation up to 70 wk animals monitored weekly by palpation for tumour development, killed when tumours persisted for ≥4 wk observation until death or killed when moribund complete autopsy, histology of skin and other selected tissues	**Mouse** Swiss mice Female (7 wk old) 40/group Control: 40 treated with the vehicle acetone alone	**DB[a,h]P** Purity: >99 % (purified by chroma-tography, followed by recrys-tallization)	119 µg Vehicle: 16.7 µL acetone 2x/wk, for a period of 30 wk	**Positive** **DB[a,h]P induced benign and malignant skin tumours after repeated dermal application of 119 µg (ca. 1.13 mg/kg bw/d)** **Tumour development on the skin:** **Tumour incidence: 89.7 %** **first tumour: after 30 wk** Number of skin neoplasms: **squamous cell papilloma: 17** **keratoacanthoma: 13** **squamous cell carcinoma: 45** **fibrosarcoma: 2** **sebaceous gland adenoma: 2** **Survival: 40 wk** All DB[a,h]P-treated mice had died or were removed by 45 weeks and 35/39 mice had skin tumours. **Control:** No skin tumours, 0/29 Survival: up to 70 weeks	Cavalieri et al. 1977

Carcinogenicity study by subcutaneous (s.c.) administration no guideline Dose group: 1 Control group: none Administration: 0.6 mg in olive oil 1 x/month, for a period of 3 months, termination: not specified	**Mouse** XVII strain, 35 males and 10 females	**DB[a,h]P** Purity not specified	0.6 mg vehicle: olive oil 1x per month, for 3 months	**Positive** **DB[a,h]P induced local sarcoma at the injection site after single subcutaneous injection of 0.6 mg per month (a total of 3 injections)** **Development of local sarcoma:** **Male: 34/35 (97 %)** mean latency: 111 days **Female: 1/10 (10 %)** mean latency: 128 days	Lacassagne et al. 1958
Carcinogenicity study by subcutaneous implantation of a paraffin disk containing DB[a,h]P no guideline Dose group: 1 no separate control group Administration: pulverized DB[a,h]P was dispersed in melted paraffin (50-52 °C), poured in a Petri disk and sterilized at 120 °C in hot air for 1h, after solidification of paraffin, disks were cut out, containing an average amount of 10 mg DB[a,h]P; were implanted subcutaneously on the right side of the chest sacrifice when tumours reached an area of about 20 mm	**Rat** Wistar Female 30/group (bw 100-110 g)	**DB[a,h]P** (pure; supplied by a supplier from UK)	100 mg/kg bw 19 months	**Positive** **100 mg/kg bw DB[a,h]P induced local sarcoma** **Tumour development on the skin:** DB[a,h]P induced sarcoma in female rats following subcutaneous implantation of a paraffin disk containing DB[a,h]P (ca. 100 mg/kg bw). From the 30 rats which had been implanted disks with DB[a,h]P in total **23 local tumours (76.6 %)** were induced after 19 months. First tumour development: after 10 months in 5 animals. At microscopy all induced tumours were identified as sarcoma.	Bahna et al. 1979

Dermal initiation–promotion study **(skin painting)** no guideline method, study protocol: to identify chemicals with promoting potential and to study the process by which normal tissue becomes a tumour 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), the skin is monitored for tumour development 9 synthesized and/or highly purified hexacyclic aromatic hydrocarbons were tested Dose group: 1 Control group: 1 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	**Mouse** Swiss albino Ha/ICR/Mil Female (7–8 wk) 29/group Control: 30/group	**DB[a,h]P** Purity: purified by chromatography, followed by recrystallization	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: 2.5 % croton oil in acetone end after 6 months	**Positive** **Significant tumour-initiating activity on mouse skin after repeated administration of 25 µg DB[a,h]P/mouse** **Tumour development on the skin:** **25 µg DB[a,h]P:** → **squamous cell papilloma** mo: months after treatment / number of mice alive / number of mice with squamous cell papilloma (in %): **2 mo / 29 / 6 (20.7 %)** **3 mo / 27 / 12 (41.4 %)** **4 mo / 27 / 17 (58.6 %)** **5 mo / 26 / 20 (69 %)** mean latency period: 90 days After 6 months squamous cell papilloma development at the injection site in nearly three quarters of the treated mice: **6 mo / 26 / 21 (72.4 %, p < 0.01)** **Control:** 2/30 (7 %) squamous cell papilloma in mice treated with the promoter 2.5 % croton oil in acetone alone	Hoffmann and Wynder 1966 LaVoie et al. 1979

Dermal initiation–promotion study **(skin painting)** no guideline (for method and study protocol s. reference Hoffmann and Wynder (1966); LaVoie et al. (1979)) Dose group: 1 Control group: 1 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 results of histology were not reported	**Mouse** CD-1 Female (50 to 55 days old) 31/group control: 32/group	**DB[a,h]P** Purity: characterized by elemental analysis, mass spectra, UV spectra, and analysis of their 270 MHz proton NMR spectra	0.2 mg in acetone 1 x, followed 1 wk later by promo-tion with 10 µg TPA in 0.1 ml acetone, 2×/wk, up to 26 wk End of the study: 27 wk	**Positive** **Significant tumour-initiating activity on mouse skin after single dose of 0.2 mg DB[a,h]P/mouse** **Tumour development on the skin** after single initiating doses of **0.2 mg DB[a,h]P** and promotion with 10 µg TPA (tetrade-canoyl-phorbol acetate): wk: weeks after treatment: tumour-bearing mice / total tumours / survivors: 10 wk: 13 / 21 / 31 15 wk: 22 / 50 / 31 20 wk: 27 / 86 / 29 **26 wk: 26 / 103 / 28 (93 %)** **Control:** 26 wk: 2 / 2 / 32 (6.25 %)	Sardella et al. 1981

Dermal initiation–promotion study **(skin painting)** no guideline (for method and study protocol s. reference Hoffmann and Wynder (1966); LaVoie et al. (1979)) Dose group: 4 Control group: 1 skin tumour development was recorded once every two weeks papilloma greater than 2 mm in diameter were included in the cumulative total when they persisted for ≥2 wk no histology results available	**Mouse** CD-1 Female (7-8 wk old) 30/group	**DB[a,h]P** Purity: 'essentially pure' on the basis of chromato-graphy, mass spectral, and nuclear magnetic resonance analysis Vehicle: 10 % DMSO	15, 60, or 180 µg in 200 µL 10 % DMSO 1 ×, followed 1 wk later by promo-tion with 10 µg TPA in 200 µL acetone, 2×/wk, 16 wk (all dose groups) 0, 15 µg up to 24 wk	**Positive** **Significant dose-related increased tumour-initiating activity on mouse skin after single doses of 15, 60, or 180 µg DB[a,h]P/mouse** **Tumour development on the skin** after single initiating dose of DB[a,h]P and promotion with 10 µg TPA: - <u>After 16-weeks:</u> Dose: % of mice with skin tumours / (corresponding numbers of skin tumours/mouse): **15 µg: 55 % / (1.41)** **60 µg: 79 % / (4.72)** **180 µg: 72 % / (5.52)** **Control:** No skin tumours - <u>After 24-weeks:</u> **15 µg: 72 % / (3.97)** **Control:** No skin tumours	Chang et al. 1982

Dermal initiation–promotion study **(skin painting)** no guideline (for method and study protocol s. reference Hoffmann and Wynder (1966); LaVoie et al. (1979)) Comparative studies of tumour-initiating activity of several dibenzo[a]pyrenes 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 tissues were fixed in 10 % buffered formalin	**Mouse** SENCAR[1] derived from SENsitivity to CARcinogenesis Female (8 wk old) 24/group control: 23/group	**DB[a,h]P** Purity: >99 % Vehicle: dioxane : DMSO (75:25)	242 µg (800 nmol) in 100 µL, 1×, followed 1 wk later by promo-tion with 2.6 µg TPA in 100 µL acetone, 2×/wk Control: dioxane/ DMSO End of study: 25 wk	**Positive** **Significant tumour-initiating activity on mouse skin after single dose of 242 µg DB[a,h]P/mouse** **Tumour development on the skin (squamous cell papilloma**) after single initiating doses of **242 µg DB[a,h]P** and promotion with 10 µg TPA: **After 25 weeks:** number of mice alive / number of squamous cell papilloma / tumour-bearing mice (in %) / number of squamous cell papilloma/mouse (calculated by dividing the total number of papilloma by the number of mice per group): **24 / 128 / 18 (75 %) / 5.3** First skin tumour: **after 10 weeks** **Control:** 23 / 2 / 2 (9 %) / 0.1 First skin tumour: **after 20 weeks**	Cavalieri et al. 1989

[1] 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).

Carcinogenicity study by intra-peritoneal (i.p.) injections no guideline Dose group: 1 Control group: 1 Application: 3 injections on days 1, 8 and 15 of life Controls: DMSO alone mice were weaned at 25 days of age tumourigenic activity was determined when mice were 49 to 54 weeks old complete necropsy, tumours were macroscopically counted, tissue samples were fixed in 10 % buffered formalin, a representative number of observed pulmonary tumours and all hepatic tumours, and all other tissues with gross lesions were examined microscopically	**Mouse** Swiss-Webster (BLU:Ha (ICR) 73 new-born mice (gender not specified) alive at weaning on day 25 in test and in control group	**DB[a,h]P** Purity: 'essentially pure' based on chromato-graphic, mass spectral, and nuclear magnetic resonance analysis	3 injections: 3.8, 7.6 and 15.1 µg (total dose of 26.5 µg) dissolved in 5, 10 and 20 µL DMSO on days 1, 8 and 15 of life Controls: DMSO alone End of study: when mice were 49 to 54 weeks old	**Positive** **DB[a,h]P induced benign and malignant tumours in lung and liver of newborn mice after 3 i.p. injections (total dose of 26.5 µg)/mouse** **<u>Lung:</u> adenomas and adenocarcinomas** (specific incidences not given) multiple nodules were found that were primary adenomatous alveolar lung tumours that arose from type 2 pneumocyte cells (also called granular pneumocytes) of the alveolar epithelium; some larger tumours have been diagnosed as adenocarcinoma with structural and cellular anaplasia, increased number of mitotic figures, and invasion in surrounding tissue (Shimkin and Stoner 1975) From **<u>14 female</u>** alive at termination (**93 %** with tumours; 4.78 tumours/mouse) From **<u>25 male</u>** alive at termination **100 %** with tumours; 5.20 tumours/mouse) **Control:** From <u>39 female</u> alive at termination: 28 % with tumours; 0.44 tumours/mouse From <u>32 males</u> alive at termination: 22 % with tumours; 0.80 tumours/mouse) **<u>Liver:</u> adenomas, sometimes accompanied by metastases to the lung** (specific incidences not given) Two hepatic tumours types were observed: (1) a simple nodular growth of the liver parenchymal cells, and (2) areas of papiliform and adenoid growth of tumour cells, sometimes accompanied by metastases to the lung (Walker et al. 1973; Wiliams at al. 1979). From **<u>14 female</u>** alive at termination **7 %** with tumours; 0.07 tumours/mouse From **<u>25 male</u>** alive at termination **44 %** with tumours; 0.88 tumours/mouse **Control:** Female: 0/39 ; Male: 0/32 **Other tumours:** Two female mice had skin sarcoma and one had an adenocarcinoma of the small intestine.	Chang et al. 1982

Carcinogenicity study by injections in mammary glands no guideline Application: single injection in each mammary gland (8) Dose group: 1 Control group: 2 animals monitored weekly by palpation for tumour development, killed when tumours ≥2 cm in diameter 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) 20/group Control: 21/group (vehicle) 20/group (no treatment)	**DB[a,h]P** >99 % by HPLC; recrystallised from xylenes	single injection of 4 µmol (1.2 µg) dissolved in 100 µL triocta-noin per mam-mary gland (8 glands total dose, 9.6 µg) control: 100 µL triocta-noin end after 40 wk	**Positive** **DB[a,h]P induced benign and malignant tumours in the mammary gland after single injection (total dose: 9.6 µg /rat)** **Tumour development in the mammary gland:** **- 19/20 (95 %) fibrosarcoma (2.4 tumours/tumour-bearing rat)** **- 4/20 (20 %) mammary adenocarcinoma (1.4 tumours/tumour-bearing rat)** **- 1/20 had mammary adenofibroma (two tumours)** **Control** - untreated group: 2/20 (10 %) mammary epithelial tumours (1 adenofibroma, 1 adenocarcinoma) - vehicle group: 0/21 (0 %) **mean survival time:** DB[a,h]P group: 30 ± 5 weeks untreated control group: 37 ± 4 weeks vehicle control group: 40 ± 0 weeks **mean tumour latency:** DB[a,h]P group: 18 ± 2 weeks untreated control group: 25 ± 13 weeks	Cavalieri et al. 1989

Dermal initiation-promotion study **(skin painting)** no guideline (for method and study protocol s. reference Hoffmann and Wynder (1966); LaVoie et al. (1979)) Dose group: 4 Control group: 1 skin tumour development was recorded once every two weeks papilloma greater than 2 mm in diameter were included in the cumulative total when they persisted for ≥2 wk no histology results available	**Mouse** CD-1 Female (51-58 days old) 30/group	**Metabolite of DB[a,h]P:** **DB[a,h]P-1,2-diol** (dibenzo[a,h] pyrene-1,2-diol) Purity: 'essentially pure' on the basis of chromato-graphy, mass spectral, and nuclear magnetic resonance analysis Vehicle: 10 % DMSO in tetrahydro-furan	17, 67, or 202 µg in 200 µL of 10 % DMSO in tetrahy-dro-furan 1 ×, followed 1 wk later by promo-tion with 10 µg TPA in 200 µL acetone, 2×/wk, 16 wk (all dose groups) 0, 17 µg up to 24 wk Control: vehicle	**Positive** **The metabolite DB[a,h]P-1,2-diol of DB[a,h]P induced significant dose-related increased tumour-initiating activity on mouse skin after single doses of 17, 67, or 202 µg/mouse** **Tumour development on the skin** after single initiating dose of the metabolite of DB[a,h]P and promotion with 10 µg TPA: - <u>After 16-weeks:</u> Dose: % of mice with skin tumours / (corresponding numbers of skin tumours/mouse): **17 µg: 39 % / (0.96)** **67 µg: 57 % / (2.73)** **202 µg: 80 % / (4.4)** **Control:** no skin tumour - <u>After 24-weeks</u>: **17 µg: 79 % / (2.96)** **Control:** no skin tumour	Chang et al. 1982

Carcinogenicity study by intra-peritoneal (i.p.) injections no guideline Dose group: 1 Control group: 1 Application: 3 injections on days 1, 8 and 15 of life Controls: DMSO alone mice were weaned at 25 days of age tumourigenic activity was determined when mice were 49 to 54 weeks old complete necropsy, tumours were macroscopically counted, tissue samples were fixed in 10 % buffered formalin, a representative number of observed pulmonary tumours and all hepatic tumours, and all other tissues with gross lesions were examined microscopically	**Mouse** Swiss-Webster (BLU:Ha (ICR) 73 new-born mice (number of gender not specified) alive at weaning on day 25 in test and in control group	**Metabolite of DB[a,h]P:** **DB[a,h]P-1,2-diol** (dibenzo[a,h] pyrene-1,2-diol) Purity: 'essentially pure' based on chromato-graphic, mass spectral, and nuclear magnetic resonance analysis	3 injections: total dose of 30 µg dissolved in DMSO on days 1, 8 and 15 of life Controls: DMSO alone End of study: when mice were 49 to 54 weeks old	**Positive** **The metabolite DB[a,h]P-1,2-diol of DB[a,h]P induced benign and malignant tumours in lung and liver of newborn mice after 3 i.p. injections (total dose of 30 µg/mouse)** **Lung: adenomas and adenocarcinomas** (specific incidences not given) multiple nodules were found that were primary adenomatous alveolar lung tumours that arose from type 2 pneumocyte cells (also called granular pneumocytes) of the alveolar epithelium; some larger tumours have been diagnosed as adenocarcinoma with structural and cellular anaplasia, increased number of mitotic figures, and invasion in surrounding tissue (Shimkin and Stoner 1975) From **28 females** alive at termination: **96 %** with tumours; 15.82 tumours/mouse From **17 males** alive at termination: **100 %** with tumours; 19.00 tumours/mouse) **Control:** From 39 female alive at termination: 28 % with tumours; 0.44 tumours/mouse From 32 males alive at termination: 22 % with tumours; 0.80 tumours/mouse **Liver: adenomas, sometimes accompanied by metastases to the lung** (specific incidences not given) Two hepatic tumours types were observed: (1) a simple nodular growth of the liver parenchymal cells, and (2) areas of papiliform and adenoid growth of tumour cells, sometimes accompanied by metastases to the lung (Walker et al. 1973; Wiliams at al. 1979). From **28 females** alive at termination: 7 % with tumours; 0.07 tumours/mouse From **17 males** alive at termination: 41 % with tumours; 3.76 tumours/mouse **Control:** Female: 0/39; Male: 0/32	Chang et al. 1982

Dermal initiation–promotion study **(skin painting)** no guideline (for method and study protocol s. reference Hoffmann and Wynder (1966); LaVoie et al. (1979)) Dose group: 4 Control group: 2 skin tumour development was recorded once every two weeks papillomas greater than 2 mm in diameter were included in the cumulative total when they persisted for ≥2 wk no histology results available	**Mouse** CD-1 Female (51-58 days old) 30/group	**Dihydrodiol epoxide, breakdown product of DB[a,h]P** racemic anti-trans-1,2-dihydroxy-1,2,3,4-tetrahydro-dibenzo[a,h]pyrene-3,4-oxide (anti-dibenzo[a,h]pyrene-1,2-diol-3,4-oxide) Purity: 'essentially pure' on the basis of chromato-graphy, mass spectral, and nuclear magnetic resonance analysis Vehicle: DMSO:te-trahydro-furan (1:10)	18, 71, or 212 µg in 200 µL of 10 % DMSO in tetrahy-dro-furan 1 ×, followed 1 wk later by promo-tion with 10 µg TPA in 200 µL acetone, 2×/wk, 16 wk (all dose groups) 0, 18 µg up to 24 wk Control: vehicle	**Positive** **The metabolic breakdown product of DB[a,h]P, dihydrodiol epoxide, induced significant increased tumour-initiating activity on mouse skin after single doses of 18, 71, or 212 µg/mouse** **Tumour development on the skin** after single initiating dose of the test substance and promotion with 10 µg TPA: - <u>After 16-weeks:</u> Dose: % of mice with skin tumours / (corresponding numbers of skin tumours/mouse): **18 µg: 33 % / (0.43)** **71 µg: 70 % / (1.87)** **212 µg: 50 % / (1.83)** **Control:** no skin tumour - <u>After 24-weeks:</u> **18 µg: 60 % / (1.00)** **Control:** no skin tumour	Chang et al. 1982

Carcinogenicity study by intra-peritoneal (i.p.) injections no guideline Dose group: 1 Control group: 1 Application: 3 injections on days 1, 8 and 15 of life Controls: DMSO alone mice were weaned at 25 days of age tumourigenic activity was determined when mice were 49 to 54 weeks old complete necropsy, tumours were macroscopically counted, tissue samples were fixed in 10 % buffered formalin, a representative number of observed pulmonary tumours and all hepatic tumours, and all other tissues with gross lesions were examined microscopically	**Mouse** Swiss-Webster (BLU:Ha (ICR) 50 new-born mice (number of gender not specified) alive at weaning on day 25 in test group 73 in control group	**Dihydrodiol epoxide, breakdown product of DB[a,h]P** racemic anti-dibenzo[a,h] pyrene-1,2-diol-3,4-oxide Purity: 'essentially pure' based on chromato-graphic, mass spectral, and nuclear magnetic resonance analysis	3 injections: total dose of 31 µg dissolved in DMSO on days 1, 8 and 15 of life Controls: DMSO alone End of the study: when mice were 49 to 54 weeks old	**Positive** **The metabolic breakdown product of DB[a,h]P, dihydrodiol epoxide, induced benign and malignant tumours in lung and liver of new-born mice after 3 i.p. injections (total dose of 31 µg/mouse)** **<u>Lung:</u> adenomas and adenocarcinomas** (specific incidences not given) multiple nodules were found that were primary adenomatous alveolar lung tumours that arose from type 2 pneumocyte cells (also called granular pneumocytes) of the alveolar epithelium; some larger tumours have been diagnosed as adenocarcinoma with structural and cellular anaplasia, increased number of mitotic figures, and invasion in surrounding tissue (Shimkin and Stoner 1975) From **<u>18 females</u>** alive at termination: **94 %** with tumours; 5.72 tumours/mouse From **<u>19 males</u>** alive at termination: **95 %** with tumours; 5.37 tumours/mouse) **Control:** From <u>39 female</u> alive at termination: 28 % with tumours; 0.44 tumours/mouse From <u>32 males</u> alive at termination: 22 % with tumours; 0.80 tumours/mouse) **<u>Liver:</u> adenomas, sometimes accompanied by metastases to the lung** Two hepatic tumours types were observed: (1) a simple nodular growth of the liver parenchymal cells, and (2) areas of papiliform and adenoid growth of tumour cells, sometimes accompanied by metastases to the lung (Walker et al. 1973; Wiliams at al. 1979). From **<u>18 females</u>** alive at termination: **6 %** with tumours; 0.06 tumours/mouse) From **<u>19 males</u>** alive at termination: **26 %** with tumours; 1.37 tumours/mouse) **Control:** Female: 0/39; Male: 0/32	Chang et al. 1982

Table 11b: Summary table of human data on carcinogenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
no report				

No case reports or epidemiological studies on the significance of DB[a,h]P exposure to man are available. However, coal-tar and other materials which are known to be carcinogenic to man may contain DB[a,h]P. 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. The data clearly suggest lung and bladder cancer. Skin cancer in man is well to known and occurs 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 PAH. Although certain occupations with high PAH exposure (e.g., coal gasification and coke production) were classified as carcinogenic in humans, the role of individual PAH could not be defined (IARC 2010).

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

DB[a,h]P is possibly carcinogenic to humans. Classification as carcinogen is largely based on animal evidence. There is sufficient evidence on carcinogenicity of DB[a,h]P. DB[a,h]P caused benign and malignant tumours in two rodent species (mouse and rat), at multiple tissue sites, and by different routes of exposure.

Evidence from animal experiments

The carcinogenic potential of DB[a,h]P has been studied in two species, mice and rats under different protocols.

DB[a,h]P was tested for carcinogenicity in three studies with topic application on the mouse skin, in a study by subcutaneous administration in mice and a study by subcutaneous implantation in rats, in four dermal tumour initiation–promotion studies in mice, by intra-peritoneal administration into newborn mice and by injection in the mammary glands of rats. DB[a,h]P exhibited significant carcinogenic activity in all of these studies.

Although the majority of the studies with DB[a,h]P were conducted decades before the EU/OECD standard test guidelines were adopted and no standard carcinogenicity study is available, it is thought that the consistency of carcinogenic action from a broad series of studies are sufficient to prove the

evidence of its carcinogenicity. The carcinogenic evidence from these studies is considered sufficiently robust to propose classification of DB[a,h]P as a carcinogen.

Repeated dermal exposure to DB[a,h]P of rather low concentrations caused benign and malignant skin tumours (squamous cell papilloma, keratoacanthoma, and epithelioma, considered as squamous cell carcinoma) in mice of both sexes. A dose-response relationship for tumour induction was observed in these studies with repeated dermal application of DB[a,h]P. In mice the lowest dose which produced squamous cell papilloma and carcinoma in females was approximately 0.86 mg/kg bw/d. Almost 90 % of the animals developed tumours within 30 weeks following dermal application of approximately 1.13 mg/kg bw/d DB[a,h]P in acetone. These tumours progressed rapidly in size, killing the host within 45 weeks.

Cancer at the site of administration (sarcoma) was observed in mice of both sexes following subcutaneous injections of DB[a,h]P and in female rats following subcutaneous implantation.

Intra-peritoneal injection of newborn mice with DB[a,h]P on three days of life caused benign and malignant lung tumours in both sexes at high incidences and benign and malignant liver tumours with lower incidence in males.

DB[a,h]P administered by single intra-mammary injection caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in female rats.

In initiation-promotion studies significant tumour-initiating activity of DB[a,h]P was demonstrated in three mouse strains.

Human data

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

DB[a,h]P is a PAH. PAH are found in many consumer products. PAH are not intentionally synthesised for use in consumer articles, but they may enter such products when certain plasticisers (‘extender oils’) or carbon black (soot) are used as additives in the production of rubber or other elastomeric materials. Inhalation and/or dermal contact are the primary routes of potential human exposure to DB[a,h]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,h]P in mice.

Germ cell mutagenicity data

DB[a,h]P induces mutagenic effects in bacteria and in exposed proliferating cells of mammalian and human cell lines, and genotoxic effects (DNA adducts) in soma cells. Combined with the read-across approach to B[a]P and CHR it is justified that DB[a,h]P may induce heritable mutations, and therefore it should be classified as category 2 mutagen in accordance with the CLP Regulation (Annex I, point 3.5.2.2, p. 145). For more details s. chapter 10.8.1 Germ cell mutagenicity.

Mode of action

No species-specific mode of action for DB[a,h]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, and posttranslational levels (epigenetic events), and altered cell survival (proliferation and apoptosis) (Hanahan and Weinberg 2000). Tumour initiation by chemical carcinogens encompasses several distinct requirements, which include the compound (reactive per se or reactive following metabolism) reacting with to reactive intermediates, their subsequent interaction with DNA and thus causing changes in DNA. In many cases these changes consist of adducts (Boström et al. 2002). In the literature it is discussed that PAH may exert both mutagenic (genotoxic) and epigenetic (non-genotoxic) actions. PAH are a class of chemical carcinogens which undergo metabolic transformations to yield active products capable of forming covalent adducts at nucleophilic sites in tissue constituents such as DNA. PAH are metabolized to reactive intermediates which bind covalently to critical cellular constituents such as DNA, RNA, and protein, causing mutations and initiating other cellular changes that result in the development of cancer (Miller et al. 1980; Miller 1970).

It has been proposed that the PAH class shares a similar mechanism of carcinogenic action. In general, PAH are converted to oxides and dihydrodiols, which in turn are oxidized to diol epoxides. Both oxides and diol epoxides are ultimate DNA-reactive metabolites. PAH oxides can form stable DNA adducts, and diol epoxides can form stable and depurinating adducts with DNA through formation of electrophilic carbonium ions (for more details s. Chapter 10.8.1 Germ cell mutagenicity).

Table 11c: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Two species and multiple strains	**Multiple tumour types**	**yes**	**yes**	**Dose-related**	**Both sexes** **males and females**	**Not noted** **(very small doses were tested)**	**Tumour induction by different routes of admin.**	**Both mutagenic (genotoxic) and epigenetic (nongeno-toxic) actions** **relevant to humans**
Mouse								
Swiss albino Ha/ICR/Mil CD-1 SENCAR derived from SEN-sitivity to CAR-cinoge-nesis	**Dermal:** benign or malignant skin tumours (squamous cell papilloma or carcinoma)	Skin tumours by topical admin.	yes	Dose-related	skin tumours in males and females	Not noted	Dermal/ skin painting/ topic	both mutagenic (genotoxic) and epigenetic (nongeno-toxic) actions
XVII strain	**subcutaneous (s.c.):** sarcoma at the injection site	Local sarcoma by s.c. admin.		No data	Local sarcoma in males and females		s.c.	
Swiss-Webster (BLU:Ha (ICR))	**Intra-peritoneal (i.p.):** benign and malignant tumours in lung and liver	lung and liver tumours by i.p. admin.		No data	lung tumours in males and females; liver tumours in males		i.p.	
Rat								
Female Wistar	**Subcutaneous (s.c.):** malignant tumours: sarcoma at the implantation site	skin tumours at the implantation site	yes	No data	only females were used Skin tumours	Not noted	s.c.	both mutagenic (genotoxic) and epigenetic (nongeno-toxic) actions
Female Sprague-Dawley	**single injection in the mammary gland:** fibrosarcoma and adeno-carcinoma	mammary gland tumours by injection in the mammary gland			only females were used Tumours in the mammary gland		single injection in the mammary gland	

10.9.2 Comparison with the CLP 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,h]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 the CLP Regulation DB[a,h]P fulfils the criteria for Category 1B carcinogen with regard to:

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

DB[a,h]P caused tumours in two rodent species, at multiple tissue sites, and by different routes of administration. Dermal exposure to DB[a,h]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, and subcutaneous implantation local tumours (sarcoma) in rats.

Intra-peritoneal injection of new-born mice with DB[a,h]P caused benign and malignant tumours of the lung in both sexes and in the liver of males. DB[a,h]P administered by intra-mammary injection has caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in female rats.

Tumours induced by DB[a,h]P have been found in the skin (by topical administration), lung and liver (by intra-peritoneal administration), and in the mammary gland (by intra-mammary gland injection).

A dose-response relationship for tumour induction was observed in studies with repeated dermal application of DB[a,h]P. In mice the lowest dose which produced squamous cell papilloma and carcinoma in females was approximately 0.86 mg/kg bw/d. Almost 90 % of the animals developed tumours within 30 weeks following dermal application of approximately 1.13 mg/kg bw/d DB[a,h]P in acetone. These tumours progressed rapidly in size, killing the host within 45 weeks.

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 nor limited to only promoting activity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

DB[a,h]P meets the criteria for classification and labelling as Category 1B carcinogen, H350 (CLP Regulation). 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,h]P and an increased incidence of a combination of benign and malignant tumours in two rodent species (mouse, rat), 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 intra-peritoneal injections in mice, and in female rats after subcutaneous implantation and injections in the mammary glands.

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

The calculation of specific concentration limits (SCL) to the carcinogen DB[a,h]P cannot be made on the basis of the available bioassays that do not follow the EU/OECD standard test guidelines using standard routes and daily dosing.

Due to the clear evidence of carcinogenic activity in experimental animals DB[a,h]P should be classified and labelled as Category 1B carcinogen, H350 (May cause cancer) in accordance with the CLP Regulation.

10.10 Reproductive toxicity

No data are available for this toxicological endpoint.

10.11 Specific target organ toxicity-single exposure

Hazard classes not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard classes not assessed in this dossier.

10.13 Aspiration hazard

Hazard classes not assessed in this dossier.

11. EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard classes not assessed in this dossier.

12. EVALUATION OF ADDITIONAL HAZARDS

Hazard classes not assessed in this dossier.

13. ADDITIONAL LABELLING

No supplemental hazard information in accordance with Annex II of the CLP Regulation.

14. DETAILED STUDY SUMMARIES

For details s. IUCLID.

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