Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

benzo[*rst*]pentaphene

EC Number: 205-877-5
CAS Number: 189-55-9

CLH-O-0000001412-86-159/F

Adopted

9 June 2017

9 June 2017

CLH-O-0000001412-86-159/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **benzo[*rst*]pentaphene**

EC Number: **205-877-5**

CAS Number: **189-55-9**

The proposal was submitted by **Germany** and received by RAC on **30 June 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **25 July 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **8 September 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 June 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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	No current Annex VI entry										
Dossier submitters proposal	601-RST-00-Y	benzo[*rst*]pentaphene	205-877-5	189-55-9	Carc. 1B Muta. 2	H350 H341	GHS08 Dgr	H350 H341			
RAC opinion	601-RST-00-Y	benzo[*rst*]pentaphene	205-877-5	189-55-9	Carc. 1B Muta. 2	H350 H341	GHS08 Dgr	H350 H341			
Resulting Annex VI entry if agreed by COM	601-RST-00-Y	benzo[*rst*]pentaphene	205-877-5	189-55-9	Carc. 1B Muta. 2	H350 H341	GHS08 Dgr	H350 H341			

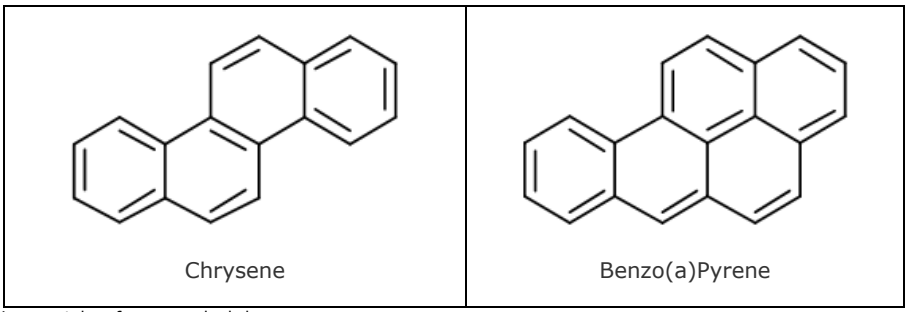
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Only two hazard classes were presented in the Dossier Submitter's (DS's) proposal for harmonised classification and labelling of benzo[*rst*]pentaphene: germ cell mutagenicity and carcinogenicity.

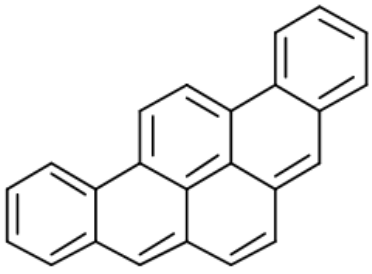
Although data from laboratory tests with this substance were presented for both hazard classes, a key supporting aspect of the proposal is its structural and biochemical similarity to other polycyclic aromatic hydrocarbons (PAHs) that are well known to possess these hazards.

There are more than 100 substances that can be termed 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 planar, highly conjugated aromatic compounds. In their report, the DS presented data on chrysene (CHR) and benzo(a)pyrene (B[a]P) which are 4- and 5- membered ring structures, respectively.



Note:images taken from www.lookchem.com

As can be seen from the following diagram, benzo[*rst*]pentaphene is structurally similar to both these substances (see below).



In addition, all 3 substances include at least 1 chemical sub-structure known as a bay region. These are the spaces that appear between the aromatic rings of these molecules. Here, metabolic oxidation can lead to the formation of dihydrodiols and subsequently to electrophilic diol epoxides. These structures 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, CHR and benzo[*rst*]pentaphene, the DS used this information in conjunction with available data to classify benzo[*rst*]pentaphene for germ cell mutagenicity and carcinogenicity.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Benzo[*rst*]pentaphene induced 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.

Clastogenic effects (induction of micronuclei) were induced by benzo[*rst*]pentaphene as well as genotoxic effects (DNA adducts, sister chromatid exchange (SCE)) in somatic tissues in both regulatory and research studies.

Amongst all the studies, only two *in vitro* mammalian cell gene mutation tests were carried out in accordance with an EU/OECD test guideline (TG); one involving mutations assayed at the TK locus, the other at the HPRT locus. Both of these tests yielded positive results.

The DS commented that the lack of appropriate controls in all the other tests was a crucial methodological shortcoming. Therefore, the results of these tests were considered as not fully reliable.

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. Consequently, even though a significant number of positive *in vitro* and *in vivo* studies had been found with benzo[*rst*]pentaphene the results of these studies alone were insufficient to support classification.

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

These PAHs have a relatively planar, highly conjugated aromatic structure. They require metabolic activation to dihydrodiol epoxides to induce genotoxic effects. 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), each with at least one reactive bay region (structural element in the PAH due to the linkage of the ring systems), are metabolised mainly through the bay-region dihydrodiol epoxides pathway. This pathway is catalysed by several enzymes such as cytochromes P450s and epoxide hydrolases. Metabolic activation finally leads to the formation of electrophilic diol epoxides that covalently bind to DNA. Benzo[*rst*]pentaphene possesses two bay regions and is one of a number of PAHs characterised structurally as relatively planar, highly conjugated six-ringed dibenzopyrenes.

The DS described a mutation test in Chinese hamster V79 cells that investigated if metabolic oxidation at the bay regions is required for the benzo[rst]pentaphene mutagenicity. Since V79 cells do not metabolise PAH, mutagenesis was tested in both the presence and the absence of hamster embryo cells capable of metabolising PAH (cell mediated assay). Having shown that a positive result could only occur in the presence of hamster embryo cells, the involvement of microsomal oxidation in the metabolic activation process was confirmed using 7,8-benzoflavone (BF), a known inhibitor of mixed-function oxidases. The mutagenicity of benzo[rst]pentaphene was prevented by additional incubation of the treated cultures with BF. To investigate whether metabolic oxidation at the bay regions was a pre-requisite for the mutagenic response, it was shown that a difluorinated derivate of benzo[rst]pentaphene (2,10-difluorodibenzo[a,i]pyrene) also inhibited the induction of mutagenic effects in this test system. This inhibitory effect implies the involvement of the particular fluorinated carbon atoms at positions of the bay regions in the metabolic activation of the parent compound. The DS thus concluded that metabolic oxidation at the bay regions was required for a mutagenic response of benzo[rst]pentaphene in this cell-mediated assay.

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

- Planar, highly conjugated aromatic structures.
- Metabolic activation required for the induction of mutagenic/genotoxic effects.
- The bay regions are common reactive centres. Dihydrodiol epoxides are formed as common breakdown products via biological processes at the bay region(s).
- Genotoxic effects induced *in vitro* and *in vivo* after metabolic activation at the bay region.

Given the limitations found in most of the mutagenicity/genotoxicity studies with benzo[*rst*]pentaphene, the DS proposed that it was justified to use the existing mutagenicity classifications of B[a]P (Cat. 1B) and CHR (Cat. 2) to support the classification of this substance. The studies showing mutagenicity/genotoxicity in somatic cells were generally of poor quality but, combined with read-across to the other mutagenic PAHs, the DS concluded they were sufficient to justify a Category 2 classification. However, a Category 1B classification was not considered appropriate as there were no studies of the mutagenic/genotoxic effects of benzo[*rst*]pentaphene in germ cells available.

Comments received during public consultation

Three MSCA communicated support for the proposal. They highlighted the positive results seen in the *in vitro* mutagenicity tests with benzo[*rst*]pentaphene and the structural similarity of this substance to the known mutagens B[a]P and CHR. One of these MSCA commented that the dossier lacked a critical assessment of the adequacy and quality of individual studies.

Assessment and comparison with the classification criteria

The potential mutagenicity of benzo[*rst*]pentaphene has been studied *in vitro* and *in vivo.*

Eleven *in vitro* studies were evaluated and included 5 Ames tests, 2 DNA repair tests in bacteria, 3 *in vitro* mammalian gene mutation assays and a UDS test. RAC agrees, however, that only two

of the eleven *in vitro* studies can be considered as reliable when judged against current regulatory standards. Both of these tests are mammalian gene mutation assays.

The *in vivo* mutagenic potential of benzo[*rst*]pentaphene was assessed in 2 micronucleus tests, in 3 DNA adduct formation assays and 1 SCE assay in rats. None of these studies conformed to a current regulatory standard.

Additionally, there are 4 initiator-promoter assays in mice to have been conducted with benzo[*rst*]pentaphene as the initiator. Positive initiation of tumours in these tests is indicative of mutagenic activity.

Given the structural similarity to other PAHs, particularly B[a]P and CHR, RAC agrees that information on these two PAHs can support the classification of benzo[*rst*]pentaphene.

In vitro studies

Five mutagenicity assays with standard tester strains of *S. typhimurium* were summarised in the CLH report. Although the DS commented that none of these tests conformed to current regulatory standards, they all gave positive results with benzo[*rst*]pentaphene. Like other mutagenic PAHs, positive results occurred in the presence of exogenous metabolic activation. The consistent nature of the results provides compelling evidence of benzo[*rst*]pentaphene's mutagenic potential. In support of this, positive results were also reported in 2 bacterial DNA repair tests (an SOS Chromotest and the Rec A assay).

According to the DS, only two *in vitro* mammalian gene mutation studies performed with benzo[*rst*]pentaphene conform to OECD TG 476 and were considered reliable for assessment.

Human B-lymphoblastoid cells engineered to express cytochrome P450 1A1 (CYP1A1) for PAH metabolism were exposed to benzo[*rst*]pentaphene at 0.3, 1.0, 10 and 100 ng/ml in the TK-locus gene mutation test. Mutation frequency was increased at the top two doses (MF 4.4 and 7.5, respectively) and was accompanied by cytotoxicity.

A gene mutation test (HPRT locus) in V79 cells tested benzo[*rst*]pentaphene at concentrations of 0.03, 0.1 and 0.3 µg/ml with and without metabolic activation. Mutant colonies were formed at all concentrations in a dose dependent manner following metabolic activation. Within this study, the necessity for the bay regions in benzo[*rst*]pentaphene to cause mutagenicity was tested. The two bay regions were individually and in combination fluorinated and mutagenic activity was measured. Fluorination of both bay regions was required to decrease the mutagenic activity of benzo[*rst*]pentaphene to background levels in V79 cells.

In conclusion, benzo[*rst*]pentaphene showed mutagenic potential when tested in cultured mammalian in the presence of a metabolic activation system sufficient for oxidation at the bay regions and formation of reactive epoxides. It has similar activity to that of other mutagenic PAHs, including B[a]P and CHR.

An *in vitro* rat hepatocyte UDS test was also available. This reported a negative result with benzo[*rst*]pentaphene but was limited in several respects, most notably in the use of only a single test concentration at a non-toxic level. It is unclear whether higher doses could have been tested and the result is not considered reliable.

In vivo studies

Micronucleus tests were conducted to investigate the genotoxicity of benzo[*rst*]pentaphene following intra-tracheal administration to male Sprague-Dawley rats. Bone marrow cells and spleen erythrocytes were harvested from groups of 5 male rats that received 3 injections of 0, 2.5, 5 or 10 mg/kg bw test substance within a 24-h harvesting period (0, 8 and 16 h). Similarly, lung cells were sampled from group of 6 rats. No information was provided on any clinical signs of toxicity induced by benzo[*rst*]pentaphene administration and no positive controls were included. There was no increase in micronucleus frequency seen in bone marrow cells (data not presented in the CLH report), although a "cytotoxic" response was evident at the top 2 dose levels. In contrast, a two-fold increase in micronucleus formation was seen in spleen cells taken from rats at the top dose. Also, compared to the negative control value, micronucleus frequency in lung cells from the 2 highest dose groups was increased 2.9 and 4.2 fold, respectively. No significant cytotoxicity was seen in spleen or lung cells.

No explanation was provided for the apparent tissue-specific effect in these studies. Although not performed to a regulatory standard, the results provide further supporting evidence of the mutagenic potential of benzo[*rst*]pentaphene.

The study of micronuclei in lung cells was extended to include the analysis of DNA adducts in the same tissue. A clear, single, adduct spot was found in samples from each dose group using the 32P post-labelling assay. No such adduct spot was seen in DNA isolated from control rat lung cells.

Post-labelling assays were also conducted by a different laboratory in skin and lung cells taken from mice administered a single topical dose of benzo[*rst*]pentaphene. Skin cells were sampled directly from the treated area. Unique DNA adduct spots were seen on autoradiographs from treated animals providing strong evidence that this PAH can produce DNA adducts in mice.

The study that found increased micronuclei and unique DNA adduct formation in the lung cells of rats also described a dose-dependent increase in SCEs. Although a rather non-specific marker of mutagenic potential, these findings were consistent with those for the other markers.

The weight of evidence from all these *in vivo* studies is strongly suggestive of benzo[*rst*]pentaphene mutagenic potential.

There were also 4 dermal initiation-promotion studies in mice with benzo[*rst*]pentaphene 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. The initiation stage of these assays is strongly indicative of mutagenic activity and the positive responses with benzo[*rst*]pentaphene support the outcomes of the *in vivo* and *in vitro* genotoxicity studies.

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

Benzo[*rst*]pentaphene shares structural properties with the PAHs CHR and B[a]P, both of which already carry a harmonised classification for germ cell mutagenicity. Notably, all 3 substances possess planar, highly conjugated aromatic structures and common reactive centres called bay regions. They all require metabolic activation at bay regions for the induction of mutagenic/genotoxic effects. Electrophilic dihydrodiol epoxides are formed as common breakdown products via biological processes at the bay regions.

CHR and B[a]P are classified as Muta. Cat. 2 and Muta. Cat. 1B, respectively. The specific data underlying these classifications was not discussed in the CLH report, therefore, it is not possible for RAC to make a detailed comparison with the data on benzo[*rst*]pentaphene to assess whether a case for a Category 1B classification could be made. However, the proposed Category 2 classification of benzo[*rst*]pentaphene is supported by the existing classifications of CHR and B[a]P.

Classification of benzo[rst]pentaphene

There are no data on human germ cell mutagenicity with benzo[*rst*]pentaphene, 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 4 positive initiation-promotion assays in mice, which gave results indicative of the mutagenic activity of benzo[*rst*]pentaphene. In the absence of data from *in vivo* studies investigating the potential effects of benzo[*rst*]pentaphene 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.

RAC is of the opinion that the available data from studies with benzo[*rst*]pentaphene itself are sufficient to justify at least a Category 2 classification. As indicated in Annex I to the CLP Regulation, Category 2 for this hazard class can be based on positive evidence from *in vivo* somatic cell mutagenicity/genotoxicity tests (e.g. for benzo[*rst*]pentaphene: micronucleus assay, DNA adduct formation, tumour initiating activity) supported by positive results from *in vitro* mutagenicity assay (e.g. bacterial and mammalian cell gene mutation assays).

Further justification is provided by the comparison made with the known mutagens B[a]P and CHR. In accordance with the criteria in Annex I (Table 3.5.1) to the CLP Regulation, "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". This could apply even when those known mutagens have harmonised classifications of Category 1A or 1B. For benzo[*rst*]pentaphene, there are also positive indications of mutagenic potential from *in vivo* studies conducted in somatic cells, but there are no germ cell studies. The use of read-across to justify a higher classification category may therefore be possible. However, this was not considered by the DS, who simply used the existing harmonised classifications of B[a]P or CHR to justify a Category 2 classification of this substance.

Overall, the RAC recommendation is for a **Category 2 classification (H341) for germ cell mutagenicity**. A Category 1B classification may alternatively be appropriate, but the relevant supporting data from the structurally similar substance B[a]P have not been provided to RAC.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of benzo[*rst*]pentaphene has been studied in numerous studies in three species under different protocols. Although the majority of these studies were conducted decades before 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.

In laboratory animals, significant tumour rates induced by benzo[*rst*]pentaphene have been found in the skin (by repeated topical administration, and a single subcutaneous injection), in the upper respiratory tract and in the lung (by intra-tracheal instillation), in the lung and liver (by intra-peritoneal administration), and mammary gland (by single intra-mammary injection).

Benzo[*rst*]pentaphene was tested for carcinogenicity in the respiratory tract in two studies with intra-tracheal instillation into hamsters. Tumour development in the upper respiratory tract and the lungs was induced in males and females after weekly instillation of low intra-tracheal doses were administered.

The long-term studies have shown induction of skin papilloma and epithelioma (considered as squamous-cell carcinoma) after repeated dermal application in mice and hamsters and increased sarcoma after single subcutaneous injection; in addition it was noted that transfer of injection-site tissues to secondary hosts shortened the latent period for producing tumours.

Three intra-peritoneal injections of benzo[*rst*]pentaphene to newborn mice caused benign and malignant lung tumours in both sexes and benign and malignant liver tumours in males. Administration by single intra-mammary injection caused cancer of the mammary gland (fibrosarcoma and adenocarcinoma) in female rats. In contrast, none of the female mice receiving benzo[*rst*]pentaphene by single intrauterine injection developed treatment-related tumours.

In addition, benzo[*rst*]pentaphene was tested for tumour initiating potential in four initiation–promotion studies on mouse skin. All four studies reported positive responses by the increased frequency of skin papilloma when compared to negative controls.

No data are available in humans exposed to benzo[*rst*]pentaphene itself. There are a number of epidemiological studies that show increased incidences of cancer in humans exposed to mixtures of PAH. Most of the PAHs have been shown to be initiators of skin and lung cancers. This feature was also provided for benzo[*rst*]pentaphene in several studies in mice and in hamsters. No species-specific mode of action for benzo[*rst*]pentaphene carcinogenesis has been identified.

There is some direct evidence that benzo[*rst*]pentaphene can be activated metabolically to the diol epoxide. Benzo[*rst*]pentaphene was metabolised to the proximate bay-region diol, dibenzo[a,i]pyrene-3,4-diol, by rat liver preparations. Dibenzo[a,i]pyrene-3,4-diol was mutagenic to bacteria and was a tumour initiator in mouse skin. It induced pulmonary and hepatic tumours in newborn mice. Although DNA adducts from antidibenzo[a,i]pyrene-3,4-diol-1,2-oxide have not been identified, synthetic anti-dibenzo-[a,i]pyrene-3,4-diol-1,2-oxide was genotoxic in bacteria and mammalian cells in culture, was a tumour initiator in mouse skin and induced pulmonary and hepatic tumours in newborn mice.

The DS concluded that benzo[*rst*]pentaphene has carcinogenic properties that justify a harmonised classification and labelling as Carc. 1B, H350.

Comments received during public consultation

Three MSCA communicated their support for the proposal. They observed how benzo[*rst*]pentaphene had been found to induce cancer at various sites in different animal species and strains, and by different routes of exposure.

Assessment and comparison with the classification criteria

There are no standard, regulatory studies to inform on the carcinogenicity of benzo[*rst*]pentaphene, but there were studies in mice and/or hamsters by the intra-tracheal, dermal and subcutaneous routes. Other less conventional studies include intra-peritoneal

administration, injection into rat mammary gland and mouse uterus. In addition, tumours induced by benzo[*rst*]pentaphene have been transplanted into secondary hosts by injection.

Dermal studies

Of all the available dermal studies, only one included concurrent control animals. In this study, which was conducted in the 1950s, female Swiss mice were administered solvent alone (p-dioxan), 0.05 or 0.1% benzo[*rst*]pentaphene three times a week for 12 months and then allowed a recovery period of 3 months. Skin papillomas and epitheliomas were reported at 0.05 and 0.1% benzo[*rst*]pentaphene (see below) but were not observed in solvent control animals.

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
	0.05% (approx. 0.86 mg/kg bw)															
Survivors	20	20	20	20	20	19	19	19	16	14	11	9	6	3	2	2
Papilloma								3	6	7	10	15	16	16	16	28
Epithelioma									3	4	6	6	9	12	13	13
	0.1% (approx. 1.71 mg/kg bw)															
Survivors	20	20	20	20	19	19	19	17	14	13	8	5	4	3	2	2
Papilloma					1	1	3	6	7	10	15	16	16	16	16	29
Epithelioma								2	4	5	8	11	12	13	15	15

At 0.1%, skin papillomas were found in 16/20 mice and skin epithelioma in 15/20 mice. In the lower dose group, skin papillomas and epitheliomas were found in 16 and 13 of the 20 test mice, respectively. The mean tumour latency period was 336 days at 0.05% and 287 days at 0.1% benzo[*rst*]pentaphene. These results provide a strong indication of the carcinogenic potential of benzo[*rst*]pentaphene.

The remaining two dermal studies, one conducted in male mice and the other in females, also reported skin papillomas, epitheliomas or carcinomas in treated animals. However, they are of limited value because they lacked concurrent controls or relevant historical control information.

Although strictly not carcinogenicity studies, 4 mouse dermal initiation-promotion studies were included in the CLH report. They appear to have been well conducted, with the inclusion of appropriate controls, and all 4 gave clear positive results for tumour formation regardless of single or multiple administrations of benzo[*rst*]pentaphene as the initiator compound.

Intra-tracheal studies

Two studies in the hamster reported respiratory tract tumours, predominantly squamous cell carcinoma, when benzo[*rst*]pentaphene was administered intra-tracheally.

In one study, 2 groups of male Syrian golden hamsters were administered approx. 2.92 mg/kg bw benzo[*rst*]pentaphene weekly over 4 weeks (total dose 8 mg) or approx. 0.73 mg/kg bw over 24 weeks (total dose 12 mg). The test substance was ground to a finely aggregated dust with haematite before administration. Control hamsters were left untreated and did not receive the vehicle (0.9% saline). After 100 weeks, all treated animals and 71/90 controls had died. Respiratory insufficiency, due to extensive tumour involvement in the respiratory tract, accounted for the increased mortality in the treated hamsters. In total, at the end of the study period, the frequency of respiratory tract tumours was 0/82, 16/34 and 39/44 in control, 8 mg and 12 mg dose groups, respectively. The majority of tumours were found in the bronchi and trachea (see below).

Dose (mg)	Number of animals with respiratory tract tumours/total (%)	Administration protocol	Number with tumour/total (%)			
			Larynx	Trachea	Bronchi	Lung
0	0/82	-	-	-	-	-
8	16/34 (47%)	2 mg fine dust with haematite resuspended in 0.9% saline and given once weekly over 4 weeks	1/34 (3%)	2/34 (6%)	13/34 (38%)	1/34 (3%)
12	39/44 (89%)	500 µg fine dust with haematite resuspended in 0.9% saline and given once weekly for 24 weeks	-	6/44 (14%)	37/44 (84%)	1/44 (2%)

Additionally, two malignant lymphomas occurred in the hamsters that received 12 mg benzo[*rst*]pentaphene. No additional tumours were seen in the 8 mg dose group. In controls, the frequency of tumours at other sites was 11/82.

Interpretation of this study is compromised by the non-physiological dose-route, which may not have been a good model for human exposure. The nature of the formulation used for dosing further complicates assessment, given that comparable solid control material was not employed. However, the results are consistent with those from the dermal studies, showing increased tumour frequency at the site of contact with the body.

A similar study was conducted in male and female hamsters (24/sex/group) at total doses of 8.5 mg (0.57 mg/kg bw/d males, 0.65 mg/kg bw/d females) and 12 mg (1.14 mg/kg bw/d males, 1.3 mg/kg bw/d females). The reported incidences of respiratory tumours was 31/48 (65%) and 36/48 (75%) at 8.5 mg and 12 mg, respectively. The majority of tumours were observed in the bronchi and trachea (see below) but tumours were present in the larynx, lung and pleura at lower incidences (specific values not given in the report). This study was limited, however, by the lack of concurrent controls or historical control data to reinforce the apparent positive outcome with benzo[*rst*]pentaphene.

Total Dose (mg)	Administration protocol	Number of animals with tumours/total (%)	% tracheal tumours*	% bronchial tumours*
8.5	500 µg in distilled water once per week over 17 weeks	31/48 (65%)	13	77
12	1 mg in distilled water once per week over 12 weeks	36/48 (75%)	19	62

* it is unclear from the CLH report by the DS whether the % is of total animals or those with tumours

Other routes of exposure

Seven mouse subcutaneous studies with benzo[*rst*]pentaphene were reported in the dossier.

In the best of these studies, groups of 12 male C57Br/cd mice were administered a single dose of benzo[*rst*]pentaphene (0.01-600 µg in peanut oil) and observed for up to 66 weeks. The incidence of fibrosarcoma at the site of injection increased with dose up to 50 µg (see below) and all animals developed tumours by week 17 in the 50, 100 and 600 µg dose groups. Palpable masses were evident by week 9 and fully developed fibrosarcomas were observed 12 weeks after injection.

Dose of Benzo[*rst*]pentaphene (µg)	Incidence of fibrosarcoma (%)
< 1	0
1	9
2	33
6.25	50
12.5	64
25	92
50	100

This study clearly demonstrates the carcinogenicity of benzo[*rst*]pentaphene administered by subcutaneous injection.

The remaining 6 subcutaneous studies in mice were all positive for local tumour formation at the site of injection. The findings of these studies support those of the study described above but they were limited by their lack of controls or short study duration. Equally, three subcutaneous studies in hamsters similarly reported fibrosarcomas at the injection site. These results support the outcomes observed in mice.

The non-conventional studies using transplantation techniques or intrauterine, mammary gland and intra-peritoneal administration were described as positive for tumour formation. In contrast, a limited study involving a single intrauterine administration of benzo[*rst*]pentaphene to mice did not evoke such a response. Given the limited reporting of these studies, the absence of suitable controls, test substance characterisation and/or detailed information about these tests for identifying carcinogenic substances, no firm conclusions can be derived from these studies.

Additional supporting information

Benzo[*rst*]pentaphene is metabolised to reactive diols via its bay regions. The proximate bay-region diol, dibenzo[a,i]pyrene-3,4-diol, was positive in an Ames test using strains TA98 and TA100 in the presence of an exogenous metabolic activation system. In a dermal initiation-promotion test, dibenzo[a,i]pyrene-3,4-diol was positive for tumour formation and produced liver and lung tumours in the mouse following intra-peritoneal injection.

Synthetic anti-dibenzo-[a,i]pyrene-3,4-diol-1,2-oxide was mutagenic in bacteria and mammalian cells without exogenous activation. In addition, tumours were formed in a dermal initiation-promotion assay and after intra-peritioneal injection in the liver and lung of the mouse.

Overall, the data provides evidence for genotoxicity and carcinogenicity via activation of bay regions; a mechanism shared with B[a]P and CHR.

Comparison with the criteria

No standard carcinogenicity studies were available with benzo[*rst*]pentaphene but there was evidence of tumour formation in a variety of tissues in multiple species with different routes of exposure. Also, the carcinogenic potential of benzo[*rst*]pentaphene is consistent with the positive genotoxicity studies and proposed classification as a mutagen.

As there is no evidence of carcinogenicity in humans with benzo[*rst*]pentaphene, Category 1A is not appropriate. There is evidence, however, of carcinogenicity in rodent species and thus Category 1B or 2 could be applied. When considering the final classification, there are many factors which can influence the outcome and in accordance with Annex I 3.6.2.2.6, RAC has compared the factors with the available data on benzo[*rst*]pentaphene from the dermal, intra-tracheal and subcutaneous routes of exposure.

Factor	Evidence with benzo[*rst*]pentaphene	Conclusion
Tumour type and background control	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	Local tumours were produced at the different sites of exposure	Tumours formed at the expected sites of exposure in humans - Cat. 1B
Progression of lesions to malignancy	Malignant tumours (fibrosarcoma, 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	Both sexes of hamster and mouse reported tumours	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 CHR which are classified as Category 1B carcinogens	Cat. 1B
Routes of exposure	Physiological (dermal) and non-physiological routes of exposure (sub-cutaneous, intra-tracheal) produced tumours	Dermal study results confirm activity after exposure by a physiological route - Cat. 1B
Comparison of ADME between test animals and humans	Not available	N/A
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	Proven 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 in Regulation (EC) 1272/2008 (see above), RAC is of the opinion that classification as **Carcinogen Category 1B (H350)** is appropriate for benzo[*rst*]pentaphene.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).