Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51

EC Number: 466-490-7
CAS Number: -

CLH-O-0000007375-70-01/F

Adopted

30 November 2023

30 November 2023

CLH-O-0000007375-70-01/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 on **30 November 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:

tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51

EC Number: **466-490-7**

CAS Number: **-**

Rapporteur, appointed by RAC: **Nina Tekpli**

Administrative information on the opinion

Sweden on **20 December 2022** 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 **30 January 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 March 2023**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51	466-490-7	-	**Add** Repr. 1B Skin Sens. 1A	**Add** H360F H317	**Add** GHS08 GHS07 Dgr	**Add** H360F H317			
RAC opinion	TBD	tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51	466-490-7	-	Repr. 1B Skin Sens. 1A	H360F H317	GHS08 GHS07 Dgr	H360F H317			
Resulting Annex VI entry if agreed by COM	TBD	tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51	466-490-7	-	Repr. 1B Skin Sens. 1A	H360F H317	GHS08 GHS07 Dgr	H360F H317			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tetra(sodium/potassium) 7-[(*E*)-{2-acetamido-4-[(*E*)-(4-{[4-chloro-6-({2-[(4-fluoro-6-{[4-(vinylsulfonyl)phenyl]amino}-1,3,5-triazine-2-yl)amino]propyl}amino)-1,3,5-triazine-2-yl]amino}-5-sulfonato-1-naphthyl)diazenyl]-5-methoxyphenyl}diazenyl]-1,3,6-naphthalenetrisulfonate;[substance having a complex composition with <80% of the above constituents and other reaction side products]; Reactive Brown 51 (hereby referred to as Reactive Brown 51) is a colorant used in textiles dyes and impregnating products.

Purity of the substance

The substance is available in the market as <80% w/w Reactive Brown 51. The concentration of other constituents present in the composition is <10% w/w. According to the Guidance for identification and naming of substances under REACH and CLP, such a composition may be regarded as corresponding to an UVCB substance and may be described under a different name. The scope of the following hazard identification relates to the composition of the substance and where Reactive Brown 51 is present as the main constituent.

For skin sensitisation, the LLNA test was conducted with FAT 40827/A which by the DS is referred to as Reactive Brown 51. The purity of the main component was approximatively 53%. The substance is described as a black sticky substance (ECHA dissemination, 2023). The vehicle used in the skin sensitisation test was dimethylformamide (DMT) which is one of the recommended vehicles described in the OECD TG 429 test guideline.

For reproductive toxicity, the reproduction/developmental toxicity screening test (OECD TG 421) was conducted with FAT 40827/B TE which by the DS is referred to as Reactive Brown 51. The purity of the main component was approximatively 55.3%. The vehicle used in the test was water.

These two studies were provided as key studies on Reactive Brown 51 by the registrant in the registration dossier. RAC is in the view that the composition of the test substance in these studies represents a typical composition of this UVCB substance and the studies are thus considered valid for the purpose of this opinion.

Toxicokinetic

Reactive Brown 51 has a relatively high molecular weight (1272.65 g/mol), a high water solubility (>306 g/L) and a low octanol/water partition coefficient (<-5.4) (ECHA dissemination, 2022).

With regard to oral administration, discoloration of urine was reported in a 28-day repeated dose oral toxicity study and in a mammalian erythrocyte micronucleus study (OECD TG 474), indicating some absorption by the gastrointestinal tract. The 28-day study also reported discoloration of faeces. These studies, together with effects reported in a reproductive/developmental screening study indicated bioavailability of Reactive Brown 51. Based on these results and the hydrophilic nature of the substance excretion is expected to be predominantly via urine and the remaining unabsorbed fraction via faeces. The substance is expected to be systemically distributed to serum because of the molecular weight and hydrophilic nature of Reactive Brown 51 (ECHA dissemination, 2022).

Overall, Reactive Brown 51 is assumed to have some degree of absorption from the gastrointestinal tract after oral administration. No data were presented on absorption by the dermal and inhalation route. However, based on the physicochemical properties of the substance

(relatively high molecular weight, high water solubility and low octanol/water partition coefficient) it is assumed to be absorbed to a limited extent via inhalation and to have low dermal uptake (ECHA dissemination, 2022).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

One Local Lymph Node Assay (LLNA) OECD TG 429 was assessed (Anon., 2006). The study was performed under GLP. No clinical signs of toxicity, systemic effects on body weight were observed during the study. Draining lymph nodes were 2-4-fold larger in the treated animals compared to control animals.

***Table**: Summary of the LLNA study by Anon., 2006 (adapted from Table 7 of the CLH report):*

Method, species, sex, no/group	Test substance and dose levels	Results
LLNA OECD TG 429 (GLP) Mouse (CBA), female, 4 animals/dose	FAT 40827/A (Reactive brown 51), purity of main component ~53%. Concentration: 2.5%, 5% and 10% Vehicle: dimethylformamide Positive control: hexylcinnamaldehyde in acetone: olive oil, 4:1	EC3 was not possible to determine. Stimulation index (SI) was 10.9, 16.8 and 23.0 at concentrations 2.5%, 5% and 10%, respectively. Positive control had a EC3 of 10.5% and was considered a skin sensitiser.

The results from the LLNA test shows that Reactive Brown 51 (FAT 40827/A) is a skin sensitiser. The stimulation index (SI) was 10.9, 16.8 and 23.0 at concentrations 2.5%, 5% and 10%, respectively. All SI were larger than 3 so it was not possible to set an EC3, however it can be assumed that the EC3 concentration would be ≤2% and the criteria for classification as Skin Sensitiser 1A is met.

Comments received during consultation

Two comments were received from member state competent authorities (MSCA) supporting classification as Skin Sens. 1A, H317.

Assessment and comparison with the classification criteria

The criteria for classification as Skin Sensitiser category 1A are met for substances showing a high frequency of occurrence in humans and/or a high potency in animals. Severity of reaction may also be considered. For the LLNA test the criteria for subcategorization 1A is an EC value ≤2%.

Reactive Brown 51 was tested in a reliable LLNA study (GLP compliant OECD TG 429). The substance is clearly skin sensitising with SI of 10.9, 16.8 and 23.0 at concentrations 2.5%, 5%

and 10%, respectively. All SI were above 3, so an EC3 could not be set. However, the data clearly indicate that the EC3 is likely to be <2% as shown in the figure below.

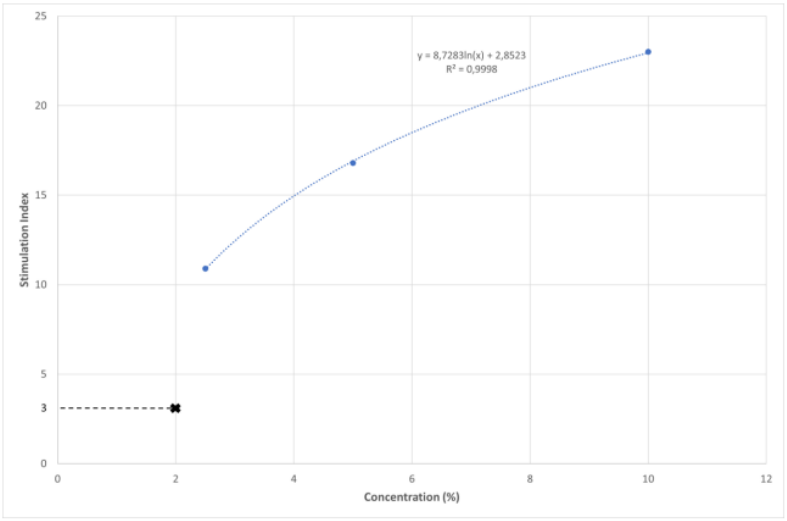

Figure from the CLH report (figure 1 in the CLH report). Log-linear regression of the data (dotted blue line) from the LLNA study and the EC3 as black dotted lines with the 2% threshold as a black cross.

Although, the substance is self-classified as skin sensitiser category 1, the registrant also reached the conclusion that the substance meets the classification criteria as skin sensitiser category 1A (ECHA dissemination, 2022 and Anon., 2013a).

Therefore**,** RAC concludes that a classification as **Skin Sensitiser 1A, H317** is warranted, as proposed by the DS.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

For the evaluation of adverse effects on sexual function and fertility, development and on or via lactation, one reproduction/developmental toxicity screening test (OECD TG 421, GLP compliant) in Wistar rats with oral gavage administration was assessed. The study was considered reliable but had some minor deviations from the test protocol (described in the RCOM, see also comments received during consultation below). In addition, a 28-day repeated dose toxicity study (OECD TG 407, GLP compliant) was used as dose-range finding study for the OECD TG 421 study.

***Table**: Summary of the adverse effects on sexual function and fertility, development and lactation in the reproduction/developmental toxicity screening test (Anon., 2013b) (adapted from table 8, 11 and 13 in the CLH report):*

Study	Test substances, doses	Results
OECD TG 421, GLP compliant Wistar rats (Han). Oral gavage administration, male/female 10 animals/ sex/group	FAT 40827/B TE (Reactive Brown 51), purity of main constituent 55.3%. 0, 100, 300 and 1000 mg/kg bw/day Vehicle: (purified) water Males: exposure 28 days prior to mating, during	There was no general toxicity at any dose. For the F0 males there were no significant reproductive effects. At necropsy a slight statistically decrease in absolute epididymis weights was observed at the highest dose. The absolute epididymis weights were 1.24, 1.24, 1.20 and 1.13 g at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The change was within the historical control range and not considered toxicological relevant. For the F0 females, reproduction was significantly affected at the highest dose (1000 mg/kg bw/day) with decreased

	mating and up to termination. Females: Exposed 41-52 days, 2 weeks prior to mating, during mating, during post-coitum and during at least 4 days of lactation.	fertility and conception indices together with decreased numbers of corpora lutea and implantation sites. 1/10 mated females showed signs of implantation (1 implantation, 2 corpora lutea). Mating index and precoital time were unaffected by treatment up to 1000 mg/kg bw/day. At 300 mg/kg bw/day the mean number of live pups at first litter check was decreased (not statistically significant). For the F1 animals there were no pups born and available for examination at the highest dose (1000 mg/kg bw/day). At 300 mg/kg bw/day there was a decrease in the mean number of live pups at the first litter check (not statistically significant) based on 2/9 litters. The mean living pups were 11.3, 11.1 and 9.8 at 0, 100 and 300 mg/kg bw/day, respectively. Historical controls 11.8. No effects on or via lactation were observed.

The dose-range finding study (Anon., 2007) was performed in Wistar rats (Han) with oral gavage administration of FAT 40827/B (Reactive Brown 51) at concentrations of 0, 50, 200 and 1000 mg/kg bw/day. 10 animals/sex for the control and high dose treated animals and 5 animals/sex for the other treatment groups. 5 animals of the control and high dose group were further observed for 14 days treatment-free recovery period. The study showed no clinical signs of toxicological relevance for any of the doses. In the males treated with 1000 mg/kg bw/day a slight reduction in body weight gain was observed during treatment and a slightly reduced body weight in the recovery period (not statistically significant). The highest dose that was used in the OECD TG 421 study was based on the lack of toxicity at the doses up to 1000 mg/kg bw/day.

Effects on sexual function and fertility

In the OECD TG 421 study there were no treatment related general toxicity in the parental animals. No relevant clinical signs and no relevant effects on food consumption or body weight/body weight gain were reported. There were some effects at the highest dose that were not considered to be toxicological relevant (decreased food consumption and body weight in dams were considered to be related to the lack of pregnancy, salivation was considered to be a physiological response and not a sign of systemic toxicity). Furthermore, purple staining of the tail was observed in the treated animals, this was considered to be caused by the staining properties of the substance and not toxicological relevant.

In males there were no signs of adverse effect on reproduction. There was a statistically significant decrease in absolute epididymis weight at 1000 mg/kg bw/day, but this change was considered to be within the historical control range.

In females, clear adverse effects on fertility were observed at 1000 mg/kg bw/day. Only one out of ten mated females became pregnant and had one implantation and two corpora lutea, however, with no live pups delivered.

Effects on development

In the OECD TG 421 study, no maternal toxicity was observed. At the highest dose, no pups were born, this was related to effects on implantation and reduced number of corpora lutea and related to sexual function and fertility as described above and not development. Since there were no live pups, developmental effects could not be assessed for the highest concentration. There were no adverse effects on development identified in the study. There was a slight, non-significant reduction in number of live pups at 300 mg/kg bw/day. However, these effects were, if treatment related, considered to be due to reduced number of implantation and more likely due to effects

on fertility. There were no effects on early postnatal development (mortality, clinical signs, body weight and macroscopy) observed up to 300 mg/kg bw/day.

Effects on or via lactation

There were no effects on or via lactation in the OECD TG 421 study. No other studies were available.

Comments received during consultation

Two comments were received from member state competent authorities (MSCA) supporting classification as Repr. 1B; H360F.

One of the MSCA had some additional questions and asked for more detail on effects on salivation, body weight gain, haematology and clinical biochemistry parameters and on behaviour of the animals receiving 1000 mg/kg bw/day in the 28-day study (OECD TG 407) to be able to conclude on general toxicity. An overview on the study data on body weights, haematology, clinical biochemistry parameters, locomotor activity was provided by the DS and is included in the RCOM. The DS as the study author consider the effects to be incidental or of such magnitude that they are considered of low toxicological relevance. RAC agrees with this assessment. Effects on salivation were only reported in the OECD TG 421 study and were considered to be a physiological response rather than a systemic effect due to low severity and occurrence after dosing.

The same MSCA also commented that the annex to the CLH report described some minor deviations from the test guideline for the reproductive reproduction/developmental Toxicity screening test (OECD TG 421) and requested some more information on the type of deviations[1]. The DS as the study author considered that the deviations did not significantly affect the integrity of the study and RAC agrees with this assessment.

Assessment and comparison with the classification criteria

No human data was available and assessment of adverse effects on sexual function and fertility and development and a classification as Repr. 1A is not justified.

According to the criteria for classification in category 1B the classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

[1] *The DS described the deviations in the RCOM as follows: formulation samples were not transferred to the weighing room but dispatched directly to the test site (samples received in good order), lack of registration of the clinical observation on LD 3 for one pup, necropsy of a dam that failed to deliver was performed on day 28 post-coitum instead of day 25-27 post coitum, and a few (common) abnormalities of kidneys and liver that was not subjected to histopathology (three control animals, one low dose and one mid dose).*

Adverse effects on sexual function and fertility

Only one reproductive toxicity study was available, a reliable reproduction/developmental toxicity screening test (GLP compliant OECD TG 421). There were no signs of general toxicity in males or females in the study and, therefore, the effects on sexual function and fertility are considered to be the primary effect of the substance.

***Table**: Summary of reproduction data in parental females (Table 10 in the CLH report)*

	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Females paired	10	10	10	10
Females mated	9	10	10	10
Pregnant females	9	10	9	**1**
Females with implantation sites only	0	1	0	1
Corpora lutea	14.8 ± 2.2	14.4 ± 2.3	13.6 ± 2.6	**2.0**
Implantations	12.7 ± 2.1	10.9 ± 4.1	11.1 ± 3.4	**1**
Mating Index (%)[1]	90	100	100	100
Fertility Index (%)[2]	90	100	90	**10**
Conception Index (%)[3]	100	100	90	**10**

[1] = (Females mated/females paired)*100, [2] = (pregnant females/females paired)*100, [3] = (pregnant females/females mated)*100

Adverse effects on fertility were reported in the OECD TG 421 study with oral (gavage) administration at the highest dose group (1000 mg/kg bw/day), summarized in the table above. Only one of the 10 mated females became pregnant and no live pups were delivered. This female showed signs of implantation with one implantation and two corpora lutea that may indicate a disruption of reproduction at fertilisation or ovulation. Mating index was unchanged.

A dose related decreasing trend was observed for the number of corpora lutea, but not significant for the 100 and 300 mg/kg bw/day dose groups. In the 100 mg/kg bw/day dose group a decreased number of implantations was also reported for two females, but with no effects on number of live pups and the finding was not considered toxicological relevant. At the 300 mg/kg bw/day dose group, it was reported a reduced number of live pups at the first litter check (9.8 in the 300 mg/kg bw/day group compared to 11.3 in the controls and 11.8 in the historical controls). The effects were not statistically significant but should be considered relevant, as related effects on fertility were observed at 1000 mg/kg bw/day. The finding was related to two females that gave birth to a lower number of pups. The same females also showed reduced number of implantations and this is in line with the effects observed at 1000 mg/kg bw/day.

Overall, the data shows clear effects on sexual function and fertility at the highest dose in the absence of adverse general toxicity. The adverse effects observed were reduced number of pregnant females, no live pups delivered, reduced females with implantation sites, reduced number of corpora lutea, reduced fertility and conception index.

Therefore, RAC concludes that **a classification for Reproductive Toxicity, sexual function, and fertility in category 1B (Repr. 1B; H360F),** is warranted, as proposed by the DS.

RAC considers that no SCL is warranted as reproductive effects (i.e. conception and fertility index are 10% reduced at 300 mg/kg bw/day) are in the medium potency range and will correspond to a generic concentration limit of 0.3% for category 1 reproductive toxicants (as proposed by the DS).

Adverse effects on development

Only one reproductive toxicity study was available, a reliable reproduction/developmental toxicity screening test (GLP compliant OECD TG 421). There were no signs of general toxicity in males or females. No pups were born at the highest dose, this was related to the lack of corpora lutea and implantations in mated females and should be considered under assessment of sexual function and fertility, not development. Since there were no pups born at the highest dose, it was not possible to evaluate developmental effects at that dose. At 300 mg/kg bw/day a lower

number of live pups were counted at first litter check when compared to control (not significant). The effects were as described above, if treatment related, most likely be a result of effects on fertility (implantation). Overall, there were no adverse effects on development that is considered relevant for classification.

In conclusion, RAC agrees that there are **no effects relevant for classification on development**, as proposed by the DS.

Adverse effects on lactation

There were no effects on or via lactation in the OECD TG 421 study. However, at the highest dose there were no pups born and therefore no pups to be examined. No effects were observed up to 300 mg/kg bw/day on early postnatal pup development and there were no examinations of pups after PND 5-7. No other studies were available.

In conclusion, RAC agrees with the DS on **no classification** based on the data available for effects via lactation.

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 and additional information (if applicable).

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