TESTING PROPOSAL ON VERTEBRATE ANIMALS: OECD 489 in vivo ALKALINE COMET ASSAY

NON-CONFIDENTIAL NAME OF SUBSTANCE:

- Name of the substance on which testing is proposed to be carried out: Direct Black 22

EC: 939-382-7

Chemical name: Reaction products of diazotised 4,4-diaminodiphenylamine-2-sulfonic acid, subsequently coupled with 6-amino-4-hydroxynaphthalene-2-sulfonic acid, further diazotised and coupled with metaphenylendiamine, sodium salts

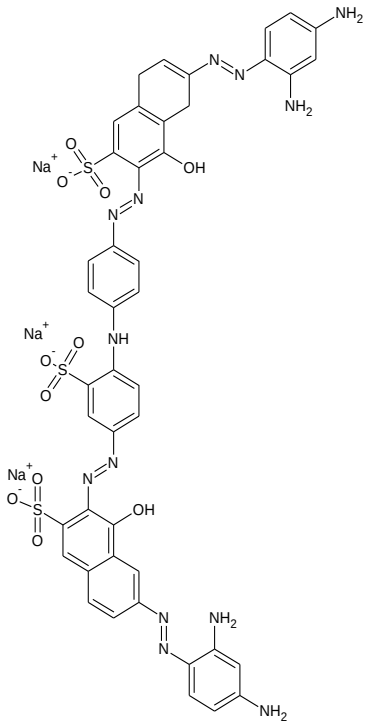
The substance is a UVCB and the main constituent correspond to the former EC.

FORMER EC: 229-326-3, FORMER CAS: 6473-13-8

Chemical name: Trisodium 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl) azo]-1-hydroxy-3-sulphonato-2-naphthyl]azo]phenyl]amino]-3-sulphonatophenyl]azo]-4-hydroxynaphthalene-2-sulphonate

Chemical formula: C44H32N13Na3O11S3

Chemical structure of the former EC:



CONSIDERATIONS THAT THE GENERAL ADAPTATION POSSIBILITIES OF ANNEX XI OF THE REACH REGULATION ARE NOT ADEQUATE TO GENERATE THE NECESSARY INFORMATION

- Available GLP studies

- Available non-GLP studies:

OECD Guideline 471 (Bacterial Reverse Mutation Assay) (S. typhimurium Strain TA 1538, TA 1537, TA 98 and TA 100)

- Historical human data: Not Available

- (Q)SAR: Not Available

- Grouping and read-across:

Some read across data available for finalisation of the genotoxicity potential
Direct Black 19, OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test)
Direct Black 19, OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)
Direct Black 168, OECD Guideline 473 (In Vitro Mammalian Chromosome Aberration Test)
Direct Black 168, OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)
Direct Black 168, OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo)
Basic Brown 1, OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo)
See genotoxicity report for other tests used in the assessment but not inserted in the dossier.

CONSIDERATIONS THAT THE SPECIFIC ADAPTATION POSSIBILITIES OF ANNEXES VI TO X (AND COLUMN 2 THEREOF) OF THE REACH REGULATION ARE NOT ADEQUATE TO GENERATE THE NECESSARY INFORMATION:

Under Annex VIII Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an in vitro gene mutation study in bacteria.

Guidance on information requirements R7a, section 7.7.6 (2017), states that REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII.

Regarding Annex VIII, when both the mammalian cell tests are negative but there was a positive result in the bacterial test, it will be necessary to decide whether any further testing is needed on a case-by-case basis. For example, suspicion that a unique positive response observed in the bacterial test was due to a specific bacterial metabolism of the test substance could be explored further by investigation in vitro. Alternatively, an in vivo test may be required

The present dossier contains different results on the target substance for the in vitro gene mutation study in bacteria following OECD 471. There are two Ames test on Direct Black 22 was conducted according to OECD Guideline 471, and it was tested in absence and in the presence of a reductive hamster liver metabolic activation system (S-9). The first test is a publication which reports positive results. The test is disregarded due to the missing details about the positive results and the lacking of information about chemical composition. The second experimental report showed negative results but only the TA98 was tested with and without metabolic activation. In any case, these results were considered as a possible evidence of Direct Black 22 mutagenic activity.

Annex VIII, Column 2 requires the registrant to consider appropriate mutagenicity in vivo studies already at the Annex VIII tonnage level, in cases where positive results in genotoxicity studies have been obtained, which involves studies mentioned in Annex IX (as first step OECD 474.Mammalian Erythrocyte micronucleus test, OECD 488 Transgenic Rodent Mutation Assay, OECD 489 In vivo mammalian Alkaline Comet Assay and OECD 486 Unscheduled DNA Synthesis).

CONSIDERATIONS ON THE STUDIES INSERTED IN THE PRESENT DOSSIER AND EXPERT ASSESSMENT ON TESTING PROPOSAL

In the present dossier two OECD 474 (Mammalian Erythrocyte micronucleus test) in vivo studies is available on analogue substances with negative results, which is adequate to cover the chromosomal aberration potential of the substance. Moreover, two OECD 486 (in vivo UDS assay) are also presented in read across from analogue substances, which resulted negative and can be used as supporting information for the gene mutation properties, since the cells analysed in the UDS assay involve only those of the liver.

The available data are reported in the table below:

OECD, substance in RA, risultati

Inserire anche Ames

Method	Substance	Results
OECD 471	Direct Black 22	Positive
OECD 474	Direct Black 19	Negative
OECD 474	Direct Black 168	Negative
OECD 486	Direct Black 168	Negative
OECD 486	Basic Brown 1	Negative

Based on the available information on gene mutation and in order to further and completely assess the gene mutation properties of the substance in different tissues of the animal a Comet Assay, OECD 489, on the target substance is presented as testing proposal.

OECD 489 allows to measure DNA strand breaks, that may result from direct interactions with DNA, alkali labile sites or as a consequence of incomplete excision repair. Therefore, the alkaline comet assay recognises primary DNA damage that would lead to gene mutations and/or chromosome aberrations, but will also detect DNA damage that may be effectively repaired or lead to cell death. The comet assay can be applied to almost every tissue of an animal from which single cell or nuclei suspensions can be made, including specific site of contact tissues.

OECD 488 is not considered the first choice for assessing the gene mutation in vivo for this substance, since preliminary data for gene mutation in vivo (OECD 486) already indicates negativity in the somatic cells of the liver. A confirmation by the Comet assay performed over other tissues (and for azo dyes the intestinal tract is the site of major metabolism and dye/metabolites absorption[i]) would be sufficient to assess the genotoxic potential of the substance[ii].

Finally, as reported in literature, from the analysis of 91 chemicals with published data from Comet Assay and Transgenic rodent mutation assay (TGR), the comet assay appears to yield similar results to the TGR assay in liver and gastrointestinal tract (predominantly stomach and colon data) and, hence, can be confidently performed to confirm in vivo gene mutation activity in terms of genotoxicity in general. [iii]

[i] Levine WG, Metabolism of azo dyes: implication for detoxication and activation, Drug Metab Rev. 1991; 23(3-4):253-309

[i] Golka et Al., Carcinogenicity of Azo Colorants: Influence of Solubility and Bioavailability, Toxicology Letters 151(1, July 2004, 203-10

[i] Yoshida.O., and Miyakawa.M. (1973), Etiology of bladder cancer: metabolic aspects, in Nakahara.W., Wirayoma.T., and Nishioka.K. (eds.), Analytical and Experimental Epidemiology of Cancer, Baltimore Univ. Park Press, pp. 31-39.

[i] Chung KT, Fulk GE, Egan M. Appl Environ Microbiol. 1978;35:558–562

[i] Chung KT, Stevens SE Jr, Cerniglia CE , The reduction of azo dyes by the intestinal microflora., Crit Rev Microbiol. 1992; 18(3):175-90

[i] Chung K-T. Environ Carcino & Ecotox Revs. 2000:C18, 51–74

[i] Cerniglia CE, Freeman JP, Franklin W, Pack LD Metabolism of azo dyes derived from benzidine, 3,3'-dimethyl-benzidine and 3,3'-dimethoxybenzidine to potentially carcinogenic aromatic amines by intestinal bacteria. Carcinogenesis.1982 Aug 31;107(4):1224–1229

i Xu, H.; Heinze, T.M.; Donald D. Paine, D.D.; Cerniglia, C.E.; Chen, H. (2010). Sudan azo dyes and Para Red degradation by prevalent bacteria of the human gastrointestinal tract. Anaerobe, Vol. 16, No. 2, pp. 114-119, ISSN 1075-9964

i Brantom PG (2005) Review of some other dyes with current non-food uses. EFSA J 263:41–71

i Wang RF, Chen H, Paine DD, Cerniglia CE, Microarray method to monitor 40 intestinal bacterial species in the study of azo dye reduction, Biosens Bioelectron. 2004 Nov 1; 20(4):699-705

[i] C N Martin, J C Kennelly, Rat liver microsomal azoreductase activity on four azo dyes derived from benzidine, 3,3'-dimethylbenzidine or 3,3'-dimethoxybenzidine, Carcinogenesis, . 1981;2(4):307-12

[i] Kennelly, J.C.; Hertzog, P.J.; Martin, C.N. (1982). The release of 4,4’-diaminobiphenyls from azodyes in the rat. carcinogenesis, Vol. 3, No. 8, pp. 947–951

[ii] https://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_genotoxicity_en.pdf/56ab5788-0103-4716-8903-59ab0c942efe

[iii] Mutat Res Genet Toxicol Environ Mutagen, . 2019 Mar;839:21-35.