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: Acid Brown 75
-EC: 232-380-0
-CAS: 8011-86-7

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

-Available GLP studies: see table below
-Available non-GLP studies: Not Available
-Historical human data: Not Available
-(Q)SAR: Not Available
-Weight of evidence: available results from an invitro study(GLP) was taken into account, that triggers the application of in vivo test listed in Annex IX
-Grouping and read-across: Read across are available to support the choice of the in vivo study to be performed

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 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 positive results for the in vitro gene mutation study in bacteria in read across from analogue substance, following OECD 471 which raises the concernfor gene mutation.

As presented in the attached document regarding genotoxicity, a fist attempt on elucidating the effect of mechanism of the nitroreductase in the positivity of the Ames test was evaluated on the target substance. The test item was tested in a modified Ames test following OECD 471 with NR deficient strains, namely TA98 and TA100, in a Prival method. The results really support the major role played by the nitroreductase and a great decrease on the number of revertants with the respect to the control was obtained for TA98 NR and TA100NR. However, the modified Ames test cannot be finalised as totally negative.

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 IN VIVO STUDIES INSERTED IN THE DOSSIER AND EXPERT ASSESSMENT ON TESTING PROPOSAL

In the present dossier an OECD 486 (in vivo UDS assay) is presented in read across from analogue substance, 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.

Moreover, an OECD 474 (Mammalian Erythrocyte micronucleus test) in vivo study is available on the analogue substance with negative results, which is adequate to cover the chromosomal aberration potential of the substance. The following table summarises the whole situation for the available tests for the genotoxicity endpoint:

test	results
In vitro gene mutation in bacteria OECD 471	POSITIVE (RA)
In vitro gene mutation in bacteria OECD 471 with NR deficient strains	Slightly POSITIVE
In vitro gene mutation in mammalian cells	NEGATIVE (RA)
In vitro cytogenicity in mammalian cells	NEGATIVE (RA)
In vivo micronucleus OECD 474	NEGATIVE (RA)
In vivo UDS OECD 486	NEGATIVE (RA)
In vivo Comet Assay OECD 489	SUBMITTED TESTING PROPOSAL
In vivo Transgenic Rodent Assay OECD 488	Not required

As reported in the above table, based on the available information on gene mutation on Acid Brown 75 and in order to further and completely assess its gene mutation properties in different tissues of the animal, a Comet Assay, OECD 489, is inserted 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 as 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) 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]

References

[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.