

4-(Phenylazo)benzol-1,3-diamin

(CAS-Nr.: 495-54-5)

4-Phenylazophenylen-1,3-diaminmonohydrochlorid

(CAS-NR.: 532-82-1)

A) Genotoxizität:

In vitro investigations:

- Studies in bacteria:

Several studies evaluating the genotoxicity of chrysoidine in the Ames test have shown positive results with and without S-9 mix. In the strains TA 100; 1537 and 98 concentrations of 0 – 2,500 µg/plate were used (Herbold, B.A. et al., 1982). In another test with TA 100 concentrations of 0 – 80 µg/plate were used and have shown positive results only with S9-mix (Sandhu and Chipman, 1990).

Remark: There are further positive studies which do not give additional evidence.

- Studies in mammalian cells:

In an UDS test on primary rat hepatocytes with concentrations of 0 – 2.5 µg/ml the result was positive (Sandhu and Chipman, 1990).

- In vivo investigations:

Studies in *Drosophila melanogaster* in feed (1%) and by injection (1% in H₂O or oil) were both negative (Foureman et al., 1994).

A micronucleus test in mice (NMRI) with a dose regimen of 0; 12; 60 and 300 mg/kg body weight according to OECD No. 474 was negative (BASF, 1988).

In rats, an UDS test with doses of 800; 1,250 and 2,000 mg/kg orally administered in corn oil gave weak positive response at each dose level with poor dose dependency. The study was independently repeated in a second experiment with the same results (ICI, 1991).

In conclusion, since there are no studies available with evidence that the substance or a relevant metabolite reach the germ cells, a category 3 based on positive results in an assay showing interactions with DNA in somatic cells in vivo (UDS Test) is warranted.

B) Carcinogenicity

1. Human data:

A report of bladder cancer in three amateur anglers with exposure to chrysoidine-dyed maggots (Searle and Teale, 1982) stimulated reports of four further cases (Massey et al., 1984; Sole, 1984) and two case-control studies (Cartwright et al, 1983; Sole and Sorahan, 1985). A study in UK used an existing large-scale bladder cancer case-control study (over 900 pairs) and made further enquiries regarding fishing, maggots and dyes used on or in the maggots. The relative risks were 0.7 (95% CI, 0.2-2.3) based on five exposed cases for yellow maggots (ready or self-coloured) (Cartwright et al, 1983). Another study in UK was smaller (202 pairs) but showed a higher percentage of use of dyed maggots (14% of cases, 8% of controls). A three-fold excess risk was noted for the use of bronze maggots for more than five years (Sole and Sorahan, 1985). This study almost certainly included five cases from the previous case reports that stimulated the case-control studies, but this factor is unlikely to remove the statistically excess risk. Evidence for carcinogenicity in humans is inadequate (IARC, 1987).

2. Carcinogenicity studies in rats and mice:

There are no valid studies available according to nowadays standards, only a negative rat study (I) and a positive mouse study (II) with serious deficiencies:

I) Rat (oral administration):

„Maruya (1938) reported that no tumors occurred in a group of 10 rats fed a diet containing 1,000 mg chrysoidine per kg of diet for 51 – 366 days.“

(cited from IARC Monographs (1975))

II) Mouse (oral administration):

„A group of 60 male and 60 female C57BL mice was fed a low vitamin diet containing 2,000 mg chrysoidine per kg of diet for 13 months, after which a control diet was given for the remainder of their life span. Two other groups of 100 (50 males and 50 females) and 130 (60 males and 70 females) mice served as controls. In the chrysoidine-treated animals liver tumors (25 adenomas and 50 adenocarcinomas) were observed in 75/104 mice, the first tumor being observed after 10 – 11 months. Metastases to the lungs occurred in 3 animals. In the control groups 1/89 and 2/117 animals developed liver tumors without metastases to the lungs. In addition, 28/104 treated mice developed leukemias and reticulum-cell sarcomas, compared with 9/89 and 12/117 in the control groups (Albert, 1956).“

(cited from IARC Monographs (1975))

Tumor Table (mouse study)

Group	Sex	No. of animals	Liver adenoma	Liver carcinoma	Combined liver neoplasia	Metastases in the lung	Leukemia and reticulum-cell sarcomas	Leukemia and reticulum-cell sarcomas simultaneously		Other tumors	Total No. of animals with tumors
								Liver	Cancer		
I	males	51	13 25.4%	16 31.3%	29 56.7%	0	17 33.3%	6 11.7%	4 7.8%	2 3.9%	38 74.5%
	females	53	12 22.6%	34 64.1%	46 86.7%	3 5.6%	11 20.6%	4 7.5%	6 1.3%	0	48 90.5%
	total	104	25 24%	50 48%	75 72%	3 2.8%	28 26.9%	10 9.6%	10 9.6%	2 1.9%	86 82.6%
II	males	44	0	0	0	0	6 13.6%	0	0	2 4.5%	8 18.1%
	females	45	0	1 2.2%	1 2.2%	0 13.2%	6	0	0	0	3 15.5%
	total	89	0	1 1.1%	1 1.1%	0	9 10.0%	0	0	2 2.2%	15 16.8%
III	males	51	1 1.9%	1 1.9%	2 3.8%	0	3 5.8%	0	0	1 1.9%	5 9.8%
	females	66	0	0	0	0	9 13.6%	0	0	3 4.5%	13 18.7%
	total	117	1 0.8%	1 0.8%	2 1.6%	0	12 10.2%	0	0	4 3.4%	18 15.3%

Group I: Mice fed for 13 months with bread and chrysoidine, afterwards with corn

Group II: Mice fed for 13 months only with bread, afterwards with corn

Group III: Mice only fed with corn

Remark:

Based on a paper by Searle (Searle and Teale; 1984) there is reasonable doubt that the test substance used in the mouse study was authentic chrysoidine: „... chrysoidine was already considered to be carcinogenic,; However, on establishing contact with Albert, we learnt of later, unpublished experiments: although his original chrysoidine proved carcinogenic in three separate experiments, he had not been able to repeat this result using fresh chrysoidine from the factory or an authentic sample prepared by a chemist. Albert suspected carcinogenic contamination of his original chrysoidine, but he may have been supplied with more than one dye. That made by the chemist would most probably have been the dye generally named „chrysoidine“ in chemical textbooks and elsewhere (i.e., 2,4-diaminoazobenzene, colour index 11270:1) or its hydrochloride chrysoidine Y (CI 11270)

IARC Evaluation Monographs (1987): Group 3 (unclassifiable):

- Evidence for carcinogenicity to humans (inadequate)
- Evidence for carcinogenicity to animals (limited)

Assessment:

The mouse study is problematic due to a number of reasons:

- The test substance has not been characterized analytically; doubts about the authenticity of the test substance used in the study.
- The (positive) result could not be confirmed by the same laboratory with an authentic sample of chrysoidine in a repeat study.
- The age of the animals (2 – 12 months); a part of them had passed their half-life span already at study begin.
- The feed is regarded as non-sufficient, since it was not standardized, neither analyses of composition nor for contaminants were performed and it was regarded as „low vitamin diet“ (see above). Control groups and the dose group received different food.
- The range of investigation is not clear („... many organs investigated ...“), nor are diagnostic criteria described.
- No clear data exist for mortality with respect to age, date of death and mortality rate. This information is crucial for an assessment of tumor formation.
- A dose-response relationship cannot be established since only one dose used.
- The only dose administered is high (2,000 ppm = ca. 300 mg/kg bw/d); this dose equals the highest dose in the mouse MNT, where unspecific toxic symptoms were observed, and is $\leq \frac{1}{2}$ of the LD₅₀ rat p.o. Thus, an overloading of liver metabolism cannot be excluded. This – together with the inadequate diet – could also have influenced the tumor formation.

Though the administration resulted in a high liver tumor rate (57% (m) and 87% (f)) as compared with controls (0% and 3.8% (m) and 2.2% and 0% (f)) the results of the study per se cannot be assessed; in addition the results could not be reproduced by the same authors. Therefore, these data cannot be used for classification purposes.

In conclusion, missing information in the documentation on the mouse study with respect to analytical characterization of the test substance including identity and purity raise substantial doubts about the validity of this study. These doubts have significant importance since in case of contamination of the test substance with aromatic amines or other azodyes a carcinogenic effect due to these other substances cannot be excluded. These significant doubts in the context with the note of Searle & Teale (1984), stating that the identities of the test substances cannot be regarded as certified, justify the decision that the mouse study has no sufficient validity.

- The rat study, which did not show any tumors after administration of a high dose (1,000 mg/kg diet) over 51 – 336 days is inadequately documented and no clear conclusion can be drawn from this investigation.

3. Potential reductive cleavage to aniline:

With respect to a possible reductive cleavage of the azo bond, the comparison of genotoxicity data of chrysoidine with those of aniline may be helpful:

- Ames test and UDS test in vitro: Aniline negative vs. chrysoidine positive
- Micronucleus test (mouse): Aniline positive vs. chrysoidine negative

These results indicate that the metabolism of chrysoidine is not via aniline or metabolites of aniline. Thus, a conclusion from the tumorigenicity of aniline to a possible tumorigenic potential of chrysoidine is not possible.

C) Toxicity for reproduction:

1. Fertility

There are no specific investigations available nor are there any results from studies with repeat dose toxicity which could be used for a classification proposal.

Thus, a classification proposal is not possible.

2. Developmental toxicity

There are no studies available. Thus, a classification proposal is not possible.

D) Conclusions

Mutagenicity:

In vitro data in bacteria and in mammalian cells as well as an UDS test in vivo show positive results.

Thus, classification in Muta. Cat. 3; R 68 is warranted.

Carcinogenicity:

The evidence for carcinogenicity in humans is inadequate. There are no valid animal studies available: In a positive mouse study there are doubts about the purity and/or the authenticity of the substance tested in addition to major deficiencies of the test

performance; the same authors were not able to repeat the results with authentic chrysoidine. A negative rat study is regarded also as inadequate due to major deficiencies in documentation. Furthermore, a comparison of the genotoxicity of the potential reductive cleavage product aniline with chrysoidine indicates a different metabolism; thus, it is also not possible to draw a conclusion.

Based on the total weight of evidence a classification with respect to carcinogenicity is not possible.

Fertility:

Due to the absence of data a classification proposal is not possible.

Developmental toxicity:

Due to the absence of data a classification proposal is not possible.

E) References

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