- Avoids the risk that additional unexpected tests would be required in a final test rule.

The agency benefits as less resources are needed than for a formal rule-making and the data required for evaluation of possible risks are available sooner.

Scope and Results of Test Program

Drosophila *SLRL Mutagenicity*

No induction of mutations in the post-meiotic germ cells.

90 Day Subchronic Toxicity

No treatment-related effects. NOEL > 2500 mg/kg/d.

Developmental Toxicity

Rat: Dose levels 0, 500, 1000, and 2000 mg/kg/d. An unexpectedly low but approximately equivalent pregnancy rate (44–56%) was observed across all dose groups. No demonstrable maternal or developmental toxicity in rats.

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Metabolism/Pharmacokinetics

Administration by gavage led to more than 95% overall recovery of the administered dose in feces and urine. Within 24 h, 85% of the dose was recovered in the feces and *ca.* 5% in the urine, indicating rapid elimination.

Early Life Stage Toxicity, Rainbow Trout

No significant concentration-related effects. NOEL greater than 4.8 µg/l, corresponding to water solubility limit.

Conclusions

The testing program provided evidence for the safety of Disperse Blue 79:1 under the conditions of the health and environmental studies conducted. The total costs of the program, *ca.* $ 600 000 for testing, administration and legal fees, were shared among the eight participating companies in the consent agreement. Following an evaluation of the data provided the EPA concluded that no further risk management of C.I. Disperse Blue 79:1 was required.

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On the Toxicology and Metabolism of Azo Dyes

Klaus Hunger*

An increasing number of regulations has somewhat led to the false impression that azo dyes in general must be considered hazardous in particular with respect to their cancerogenic and mutagenic properties. Based on the metabolic pathways and the structural features of azo dyes an assessment of the cancerogenic potential is being made.

1. Division of Azo Dyes

Two groups of azo dyes are to be considered:

- water-soluble dyes, mostly carrying sulfonate groups
- solvent-soluble dyes with non-polar substituents.

This division is justified by the different metabolic pathways of both groups.

2. Toxicological Properties

Azo dyes in general have a low toxic profile [1], certainly of most concern is the cancerogenic potential. In fact, only a very small number of the *ca.* 3000 different azo dyes on the market are cancerogenes, and these are not produced anymore by responsible manufacturers.

An assessment of the possible cancerogenic potential of azo dyes would help to decide early in the development of new products whether to proceed or to stop and save high expenses for toxicological investigations.

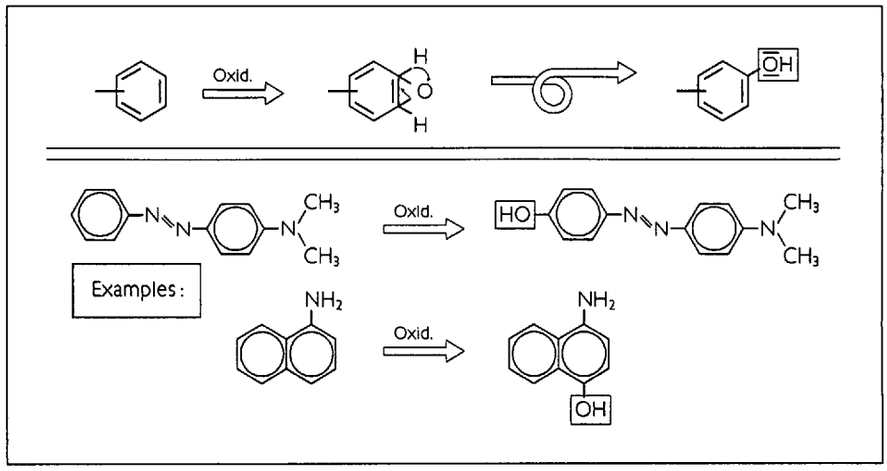
3. Metabolism

Enzymes catalyzing all metabolic reactions are unable to differentiate whether degradation products are hazardous for the organism. Oxidation and reduction reactions are the most important degradation mechanisms for azo dyes.

3.1. *Oxidative Metabolism*

Dyes with greater lipid solubility undergo preferably oxidation reactions. Oxidative processes are mainly catalyzed by

Scheme 1. C-*Hydroxylation* (ring hydroxylation)



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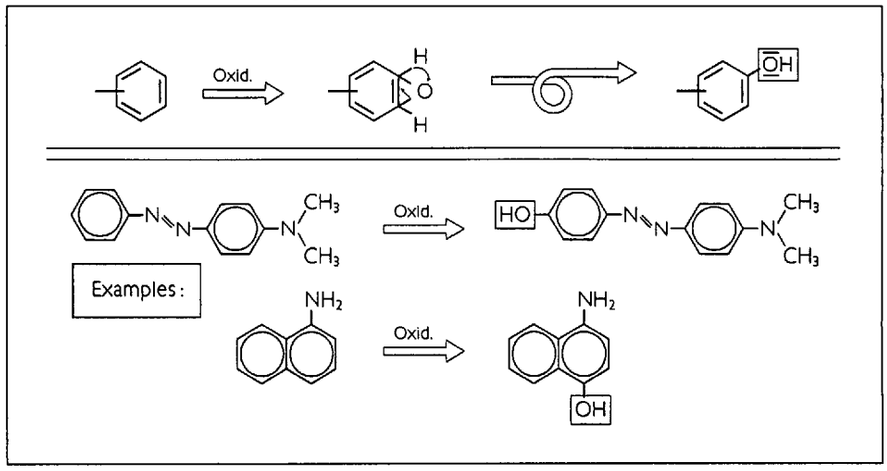

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a microsomal monooxygenase system represented by cytochrome P-540. The general metabolic oxidation mechanism works through an electron transport chain firstly transferring an electron to the P-540-Fe^{3+} complex, which upon reduction, intake of oxygen and subsequent steps finally leads to the oxidation product in the organism [2].

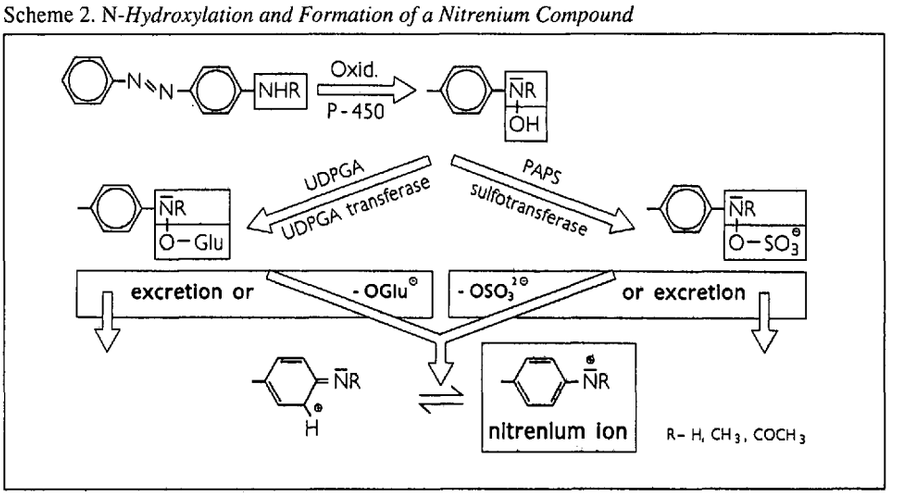
Three different oxidation pathways are important for azo dyes, all are leaving the azo bond intact:

C-*Hydroxylation*, a ring hydroxylation for azo dyes, probably proceeding *via* an epoxidation mechanism and subsequent rearrangement to phenol (*Scheme 1*).

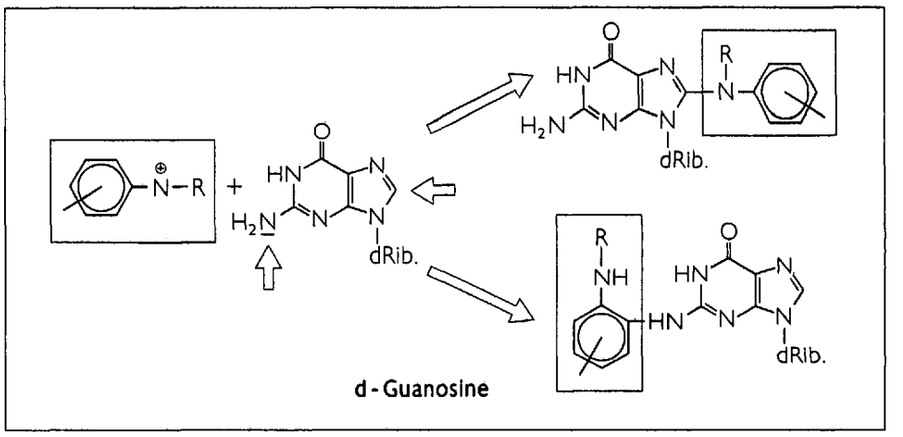
N-*Hydroxylation* occurring at primary or secondary amino groups or with acetylamino groups in the liver. This reaction is followed by an esterification with glucuronate or sulfate. These activated esters can lead to an excretion of the now water-soluble substance or it splits off the ester group with formation of a nitrenium compound $-NH^+$ (*Scheme 2*) which can covalently bind to a nucleophilic group of the DNA, as illustrated with guanosine (*Scheme 3*).

Demethylation is the stepwise oxidation of the methyl groups of dialkylamino compounds. The *N*-hydroxy derivative can be further demethylated or reacts to a nitrenium compound (*Scheme 4*).

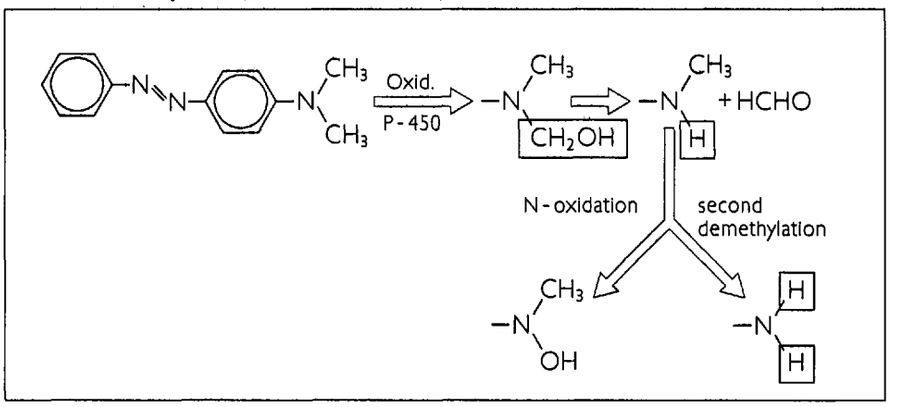
Scheme 2. N-*Hydroxylation and Formation of a Nitrenium Compound*



Scheme 3. *Reaction of the Nitrenium Compound with DNA* (guanosine)



Scheme 4. *Demethylation* (oxidative metabolism)



3.2. *Reductive Metabolism*

By far the most predominating reductive metabolic reaction on azo dyes occurs by cleavage of the azo linkage. This reduction takes place preferably with water-soluble dyes.

Azoreductase is represented by the microsomal NADPH-cytochrome P-540 system with NADPH as electron donor. The system is either found in the liver or formed by many species of anaerobic bacteria in the large intestine.

The mechanism of azoreductase in mammals involves a two-electron transfer with a free radical intermediate (*Scheme 5*). The reduction ability depends highly on the enzyme system and the substitution pattern of the azo dye. In the case of *Acid Orange 10*, electron-withdrawing groups in the 3- or 4-position of the azo bond are accelerating, whereas electron-donating groups in these positions are inhibiting the enzyme reaction as well as a sulfonate group in the *o*-position (*Scheme 6*) [3].

3.3. *Biokinetics*

There is ample evidence that water-soluble azo dyes, if orally administered, are metabolized by the intestinal microflora. In addition, these sulfonated dyes are excreted more rapidly than less soluble compounds.

Water-insoluble dyes are efficiently absorbed in the gut without bacterial reduction. Metabolic glucuronation takes place in the liver, forming water-soluble conjugates which are subsequently transported *via* the bile into the intestine where the bacterial azo reduction occurs.

Studies on the radioactive-labelled *Remazol Black B* were carried out [5]. The rapid reduction of the azo bond yielded 84% of the *p*-base ester [1]. 99% of the radioactivity were eliminated within 24 h *via* the feces. All excreted metabolites were even more polar than the dye itself. This clearly supports the assumption that neither the dye nor any of its metabolites has a bioaccumulation potential.

4. Aromatic Amines

The most characteristic metabolites of the azo bond reduction are the aromatic amines, structural features of which are

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Table. *Assessment of Carcinogenic Potential*

	Azo Dyes			
	General Properties	Solubility	Prefered Metabolic Reactions	Carcinogenic Potential
1)	Diazo component is carcinogenic (no sulfonate groups)	Water- or solvent-soluble	1. reduction of –N=N– 2. *N*-oxidation of the amine	+
2)	Azo linkage remains intact (amino group in the molecule)	solvent-soluble	ring oxidation *N*-dealkylation *N*-oxidation	+/–
3)	Both moieties carry sulfonate groups	water-soluble	reduction of –N=N– quick passing the body	–

influencing the cancerogenic potential of azo dyes as follows:

- the position of the NH_2 group. A free *p*-position allows the ring hydroxylation and subsequent detoxification of the molecule.
- Sulfonate groups in the molecule lead to high solubility, thus reducing the dwell time of the aminosulfonic acid [4]. Due to the high polarity the association with the hydrophobic region of the protein is hindered and correspondingly the transport to the cell nucleus.
- Steric hindrance of the amino groups as demonstrated with the non-genotoxic 3,3'-alkyl-(>C_3) or -alkoxy-(>OC_2) substituted benzidines [5].

5. Assessment of the Cancerogenic Potential

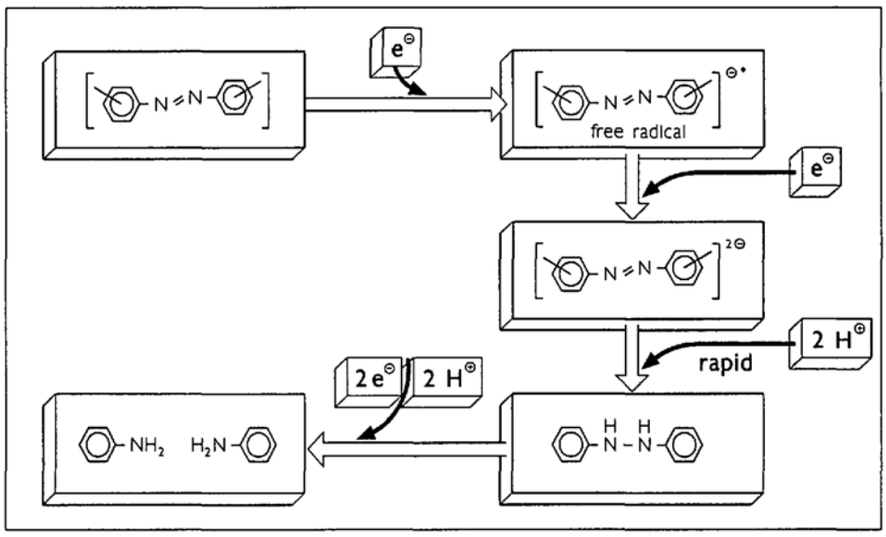
Taking into consideration the different metabolic pathways the cancerogenic potential of azo dyes can now be assessed.

Three groups should be considered: Firstly, if the reaction product with azoreductase contains a carcinogenic aromatic amine, the formation of this substance again appears to account for the carcinogenicity of the azo dye. Example: *Direct Blue 6* (benzidine).

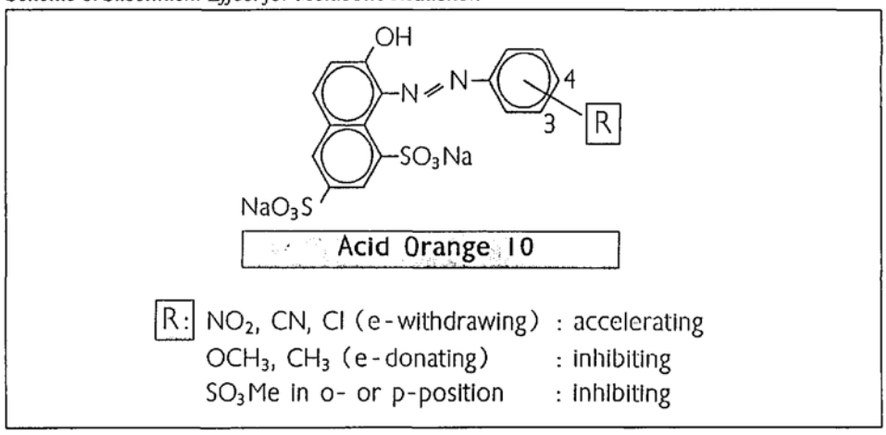
Secondly, azo reduction does not yield carcinogenic amines. The carcinogenicity of the dye itself containing amino-, alkylamino-, or acetylamino groups but no sulfonate groups may be attributable to its direct *N*-hydroxylation. Example: the hepatocarcinogen dimethylaminobenzene.

Thirdly, both moieties, *e.g.* the diazo and the coupling component of the dye carry sulfonate groups providing a high water-solubility which prohibits absorption in the organism and facilitates the elimination of the reduced but otherwise unchanged moieties of the azo dye from the body. Example: *Food Red 1*, C.I. Const. No. 14700.

Scheme 5. *Microsomal Azo Reduction Mechanism*



Scheme 6. *Substituent Effect for Metabolic Reduction*



6. Conclusion

The *Table* presents a scheme which, as a conclusion, is the assessment of the carcinogenic potential of azo dyes in relation to their chemical structure.

I like to thank Dr. *R. Jung*, *Hoechst AG*, for many fruitful discussions.

[1] K. Hunger, R. Jung, *Chimia* **1991**, *45*, 297.
[2] G.F. Fuhrmann, 'Allgemeine Toxikologie für Chemiker', B.G. Teubner, Stuttgart, 1994.
[3] M.A. Brown, S.C. De Vito, *Crit. Reviews in Environm. Sci. Technol.* **1993**, *23*, 249.
[4] R. Jung, D. Steinle, R. Anliker, *Food Chem. Toxicol.* **1992**, *30*, 635.
[5] *Hoechst AG*, unpublished, see also [1].