Mutagenicity of different textile dye products in *Salmonella typhimurium* and mouse lymphoma cells

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Abstract
European textile dye products were examined for available published and unpublished data. 53 dye products not investigated for mutagenicity up to that point were selected for testing in the bacterial reverse mutation assay with *Salmonella typhimurium* (Ames test). A modification of the OECD 471 was used (only the two strains TA98 and/or TA100 with and without metabolic activation [S9-mix] instead of five). About 28% (15 out of 53) of the dye samples were positive in the Ames test. 15 samples showed positive results with TA98, 2 with TA100. The Mutagenicity of 9 Ames positive textile dye products was further investigated in the mouse lymphoma assay (MLA) (OECD 476). 67% (6 out of 9) induced genotoxic effects in the MLA. The induction rates (IR) were between 2.1 and 132 in the bacterial reverse mutation assay and in the range between 2.1 and 15.2 in the MLA. The results confirm previous findings that dye products are marketed which are not sufficiently tested and which show mutagenic effects in *in vitro* tests.

1. Introduction

Several experimental investigations have shown that textiles and waste water from textile finishing companies (TFCs) contain mutagenic substances [1 – 5]. The systematic backtracking of the flows of waste water from the production plants of three textile processing companies led to the identification of textile dyes as a cause of the high mutagenic effects [6 – 8].

In the European Union (EU) new chemicals must be examined for mutagenic effects for notification. The base-level set requires two *in vitro* tests, usually a gene mutation test in bacteria and an *in vitro* mammalian cell test [9, 10]. Most textile dyes in use are so called ”existing substances“, which have been placed on the market before 1983. Many of them have therefore not been adequately tested until now.

In 2001 a two year CRAFT project (**C**ooperative **R**esearch **A**ction **f**or **T**echnology, a special program to support **s**mall and **m**edium sized **e**nterprises-SMEs) (QLK4-CT-2000-70158) was started with 9

TFCs and 4 research partners (RTDs; Research-Technology and development) from 8 European countries. This project aimed at the identification and substitution of mutagenic dyes in these companies and developing a general substitution strategy.

The test strategy was as follows:

- Dye products used in the different textile finishing companies and their respective amounts of consumption per year were listed. Information on dye composition and mutagenicity data for the component dyes and the formulated products was requested from the dye producers.
- Available mutagenicity data from literature were summarized and evaluated.
- Dyes without any mutagenicity data and high priority for the participating TFCs were selected for testing during the project.
- Ames tests with textile dye products were performed
- MLA where carried out with selected Ames positive dyes.

In the project dye products were tested as they are used at the TFCs. In many cases products are mixtures of different dyes and additional auxiliaries. In the following the results from the tests with 53 dye products are presented.

2. Materials and Methods

Tests for reverse mutation in the bacterium *S. typhimurium* (Ames test) [11] were carried out essentially following OECD TG 471 [12] and Commission directive 2000/32/EC, B.13/14 [13]. In deviation to the above mentioned guidelines only the two *S. typhimurium* strains TA98 and TA100 (Dr. B. N. Ames, Berkeley, CA, USA.) were applied, in presence and absence of a metabolic activation system (S9 Moltox, Boone, NC, USA). These strains are commonly used for screening of mutagenicity because they indicate both frame shift (hisD3052 with TA98) and base pair (hisG46 with TA100) mutations. They are recommended in the DIN guideline 38415-4 for the testing of waste water. Experiments with unclear results were repeated in independent studies. All tests were performed with three plates in repetition. For the cultivation of the bacteria Nutrient Broth No. 2 (Oxoid, Basingstoke, England) was used. Agar plates and soft agar were prepared with Bacto-Agar (Becton, Dickinson & Company, Sparks, U.S.A.) and Rat liver S9 Aroclor 1254 induced for metabolic activation was purchased by Moltox Inc. Boone, USA. The positive control substances (1.5 µg Nitrofluorene (NF) per plate with TA98 without metabolic activation, 0.5 µg Sodium azide (SA) per plate with TA100 without metabolic activation, 2.0 µg 2-Aminoanthracene (2-AA) per plate with TA98 with metabolic activation and 2.5 µg 2-AA per plate with TA100 with metabolic activation) were purchased by Sigma Chemical, St. Louis, U.S.A.. A sample was classified as mutagenic if it caused more than a doubling of the number of revertant colonies per plate in comparison to the control in at least one strain either with or without the metabolic activation system and/or a concentration-related increase over the range tested. Additionally the increase of the number of revertant colonies was analysed statistically using the Wilcoxon rank sum test according to Mann & Whitney [14]. Testing was performed with five concentrations between 0.5 and 5,000 µg/plate. The mouse lymphoma assay (MLA) was applied to dye stuffs which proved to be Ames positive. Tests using cell line L5178Y $TK^{+/-}$ Clone 3.7.2.C (obtained from Schering, Berlin, Germany) were performed in 96-well microtiter plates following OECD TG 476 [15] and

Commission directive 2000/32/EC, B.17 [16]. In a first test five concentrations between 15.8 and 5,000 µg/ml were tested. Further tests were performed with smaller concentration steps.
A test was evaluated as valid if the cloning efficiency (CE2) was at least 10%. A sample was evaluated as positive if the total mutant frequency (small and large colonies) was elevated more than about 100 per 10^6 surviving cells in comparison to the control in at least one concentration and if this increase was significant (one way ANOVA followed by Dunnett`s test). Small and large colonies were differentiated to assess whether genotoxic effects are due to chromosome or gene mutations [17 - 19].
Samples of the dyes were provided by the participating TFCs as they are used in their companies.

3. Results

Bacterial reverse mutation assay

53 dye products were tested in the bacterial reverse mutation assay. The highest induction rate (IR) detected in either strain is presented in table 1. All positive dye products, which had an IR ≥ 2 or with a clear dose response relationship, are marked with grey. The measured induction rates in the positive samples were between 2.4 and >132. The dose-related results of the positive samples are shown in table 2.

Mouse Lymphoma Assay

Nine Ames positive dye products were selected and tested additionally in the mouse lymphoma assay. In addition to 6 of the dyes discussed above, 3 dye stuffs for which positive Ames test results were indicated by the producing companies were chosen for testing. The IRs of samples evaluated as positive ranged between 2.2 and 15.2. The results are presented in table 3. Positive samples are marked in grey. Dose-response curves are presented in figure 1.
With some samples strong toxic effects were detected. Difficulties in the evaluation of mutagenicity encountered in these cases are exemplified with ID 148 “Astrazon Blue BG 200% 0.1“. This dye had to be investigated in three separate tests. The first assay with concentrations between 5,000 and 157 µg/ml could not be evaluated due to toxicity. In the second study, concentrations between 158 and 0.5 µg/ml were tested. With 158 µg/ml toxicity was again 100%, with 50 µg/ml the mutant frequency reached eight fold of the negative control, but toxicity was still 96%. With the next lower tested concentration of 15.8 µg/ml the mutant frequency was only slightly increased, while toxicity was reduced to 14%. Therefore in the third study concentrations between 50 and 20 µg/ml were tested. With concentrations ≥ 30 µg/ml mutant frequencies were significantly increased and toxicity was between 40 and 85%.
About 28% (15 out of 53) of the dye samples were positive in the bacterial reverse mutation assay (strains TA98 and/or TA100). Mutagenicity of 9 Ames positive textile dye products was further investigated in the mouse lymphoma assay (MLA). 67% (6 out of 9) induced mutagenic effects in the MLA (table 3). Small and large colonies were differentiated. With Erionyl Bordeaux and Turquoise Cibacrone no significant induction of large colonies was detectable. With Bleu Terasil, Astrazon Blue FGRL and Astrazon Blue BG induction of mutant frequency of large colonies was lower or in the same range as with small colonies. Only with Bemaplex Black mutant frequency of large colonies was elevated more than those of small colonies, based on the calculated induction rates.

Table 1: *List of dye products tested in the bacterial reverse mutation assay (TA98 and TA100). Positive products are marked with grey and the highest induction rate achieved is presented for the most sensitive strain*

Dye Product Name	IR*	Dye Product Name	IR*
Astrazon Red FBL	negative	Lanasol Rot B	TA100-3.6
Bemaplex Schwarz C-2B	TA98-111.0	Lanasol Gelb 4G	negative
Blanc Minerprint 51	negative	Levafix Blau E-GRN 01	negative
Bleu Cibanone 83962 MD liq	negative	Levafix Brillantrot E-BA Granulat	negative
Bleu Imperon K-RR	negative	Lumacron Black SEF 300%	TA98+41.8
Bleu Terasil 3R-02	TA98-35.5	Lumacron Red PGA	TA98+5.9
Brun Cibanone 2RMP	TA98-4.3	Noir Acramin FBB 01	negative
Brun Cibanone BR MD liq. 40%	TA98-6.6	Noir Indanthren G sfx	negative
Brun Indanthren HRR sfx	negative	Olive Cibanone 2R MD	TA98+2.4
Chromafix Black GR	negative	Orange Imperon K-G	negative
Chromafix Tyrqoise G 150%	negative	Orange Minerprint 3RL	TA98-10.7
Cibanon, Türkis P-GR	negative	Ostacelová Cerven E-LB 180	negative
Dianix gelb SE-5G	negative	Procion Blau H-ERD	negative
Erionyl Red A-2BF	negative	Remazol Black N-150	negative
Evercion Blue H-EGN 125%	negative	Rouge Imperon K-B	TA98+9.0
Evercion Blue H-ERD	negative	Rouge Terasil P3G	TA98-77.7
Evercion Navy Blue H-ER	TA98+>132	Saturnová MODR L4G 300	negative
Evercion Red H-E3B	negative	Saturnové Bordo LB 140	negative
Evercion Red H-E7B	negative	Saturnová SED LCG	negative
Evercion Yellow ESL	negative	Sirius Grau K-CGL	TA98-6.0
Evercion Yellow H-E4G	negative	Sirius Orange K-FCN	negative
Evercion Yellow H-E4R	negative	Terasil Blue 3RL-02 150%	negative
Foron Brillant Red E-2BL 200	negative	Turquoise Cibacrone P-GR Liq. 50%	TA98+2.6
Helizarin Gris BT conc. 96	negative	Vert Otan Cibanone 323 IR-01 liq	negative
Imcosol Grau 4G 200%	negative	Violet Cibacrone P-2R liq 33%	negative
Jaune or Cibanone RK MPATE	negative	Violet Imperon K-B	negative
Lanasol Red 6G	TA100-5.8		

Dye products tested positive in the Ames test are marked with grey, the highest induction rate (IR*) achieved and the corresponding strain is given.

TA98-: *S. typhimurium* strain TA98 without metabolic activation
TA98+: *S. typhimurium* strain TA98 with metabolic activation
TA100-: *S. typhimurium* strain TA100 without metabolic activation
TA100+: *S. typhimurium* strain TA100 with metabolic activation

Table 2: Dose-response results for the dye products tested positively in the bacterial reverse mutation assay

		TA98 without S9			TA98 witht S9			TA100 without S9			TA100 with S9		
sample	concentration µg/plate	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR
NC (DMSO		18	23		28	13		113	13		128	5	
Bemaplex schwarz C-2B	0.5	11	24	0.6	21	40	0.8	104	7	0.9	115	16	1.0
	5	35	13	1.9	39	10	1.4	101	13	0.9	106	6	0.9
	50	279	9	**15.2**	134	9	**4.8**	124	8	1.1	109	13	1.0
	500	795	8	**43.4**	710	15	**25.3**	255	7	**2.3**	223	13	**2.0**
	5000	2040	4	**111.3**	1651	2	**59.0**	727	5	**6.4**	521	4	**4.6**
PC *	*	440	9	24.0	203	3	7.3	469	6	4.2	531	5	4.7
NC (DMSO		24	33		32	8		150	15		124	11	
Bleu terasil 3R-02	0.5	25	5	1.1	37	26	1.1	152	5	1.0	124	5	1.0
	5	24	7	1.0	49	17	1.5	142	16	0.9	136	9	1.1
	50	71	9	**3.0**	158	7	**4.9**	153	16	1.0	131	8	1.1
	500	250	7	**10.4**	567	13	**17.5**	176	13	1.2	190	8	1.5
	5000	852	13	**35.5**	961	10	**29.7**	322	4	**2.1**	299	5	**2.4**
PC *	*	340	19	14.2	340	15	10.5	371	5	2.5	719	14	5.8
NC (DMSO		21	7		n.d.			108	4		n.d.		
Brun Cibanone 2RMP	0.5	20	16	1.0	n.d.			130	9	1.2	n.d.		
	5	19	6	0.9	n.d.			119	12	1.1	n.d.		
	50	17	9	0.8	n.d.			128	12	1.2	n.d.		
	500	31	27	1.5	n.d.			166	7	1.5	n.d.		
	5000	90	9	**4.3**	n.d.			217	15	**2.0**	n.d.		
PC *	*	355	17	17.2	n.d.			458	3	4.3	n.d.		
NC (DMSO		22	40		40	8		108	12		124	7	
Brun Cibanone BR MD liq. 40%	0.5	21	17	0.9	29	19	0.7	113	5	1.0	102	9	0.8
	5	20	42	0.9	29	21	0.7	130	12	1.2	99	8	0.8
	50	29	26	1.3	31	18	0.8	122	4	1.1	109	6	0.9
	500	52	21	**2.4**	42	17	1.1	127	6	1.2	121	14	1.0
	5000	145	10	**6.6**	115	7	**2.9**	149	13	1.4	133	13	1.1
PC *	*		9	18.2	197	2	5.0	357	9	3.3	503	7	4.1

NC: Negative Control ($H_2O_{deion.}$)
PC: Positive Control (TA98-: Nitrofluorene 1.5 µg/plate;
TA98+: 2-Aminoathracene 2.0 µg/plate;
TA100-: Sodiumazide 0.5 µg/plate;
TA100+: 2-Aminoanthracene 2.0 µg/plate)

IR: Induction Rate
(number of revertants sample / number of revertants control)
RSD: relative standard deviation of mean [%]

		TA98 without S9			TA98 witht S9			TA100 without S9			TA100 with S9		
	concentration	mean	RSD		mean	RSD		mean	RSD		mean	RSD	
sample	µg/plate	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR
NC (DMSO		30	5		30	4		150	11		134	11	
Evericon Navy Blue H-ER	0.5	29	19	1.0	28	2	0.9	126	7	0.8	131	12	1.0
	5	26	15	0.9	37	32	1.2	126	4	0.8	122	12	0.9
	50	140	5	**4.7**	543	16	**17.9**	355	7	**2.4**	814	2	**6.1**
	500	925	9	**31.2**	>4000	0	**>132**	1677	22	**11.2**	>5000	0	**>37**
	5000	2000	0	**67.4**	>4000	0	**>132**	>5000	0	**33.4**	>5000	0	**>37**
PC *	*	453	19	15.3	252	9	8.3	476	15	3.2	521	7	3.9
NC (DMSO		31	28		35	22		129	5		126	4	
Lanasol Red 6G	0.5	25	0	0.8	25	27	0.7	126	12	1.0	104	6	0.8
	5	22	9	0.7	33	18	1.0	102	10	0.8	95	7	0.8
	50	21	12	0.7	26	44	0.8	113	5	0.9	99	8	0.8
	500	32	16	1.0	27	6	0.8	171	8	1.3	110	14	0.9
	5000	171	4	**5.5**	72	11	**2.1**	743	9	**5.8**	426	6	**3.4**
PC *	*	436	11	14.1	198	9	5.7	578	10	4.5	399	10	3.2
NC (DMSO		26	10		26	21		120	4		130	9	
Lanasol Rot B	0.5	21	25	0.8	37	32	1.5	103	7	0.9	111	10	0.9
	5	15	10	0.6	29	12	1.1	109	5	0.9	132	9	1.0
	50	19	11	0.7	25	8	1.0	129	11	1.1	127	4	1.0
	500	24	25	0.9	22	9	0.9	137	12	1.1	146	7	1.1
	5000	43	27	1.7	31	20	1.2	430	4	**3.6**	362	9	**2.8**
PC *	*	401	28	15.4	200	12	7.8	501	5	4.2	451	9	3.5
NC (DMSO		n.d.			40	8		n.d.			n.d.		
Lumacron Black SEF 300%	0.5	n.d.			38	12	0.9	n.d.			n.d.		
	5	n.d.			41	8	1.0	n.d.			n.d.		
	50	n.d.			289	27	**7.3**	n.d.			n.d.		
	500	n.d.			1657	23	**41.8**	n.d.			n.d.		
	5000	n.d.			1342	10	**33.8**	n.d.			n.d.		
PC *	*	n.d.			197	2	5.0	n.d.			n.d.		

NC: Negative Control ($H_2O_{deion.}$)

PC: Positive Control (TA98-: Nitrofluorene 1.5 µg/plate;
TA98+: 2-Aminoathracene 2.0 µg/plate;
TA100-: Sodiumazide 0.5 µg/plate;
TA100+: 2-Aminoanthracene 2.0 µg/plate)

IR: Induction Rate
(number of revertants sample / number of revertants control)

RSD: relative standard deviation of mean [%]

		TA98 without S9			TA98 witht S9			TA100 without S9			TA100 with S9		
	concentration	mean	RSD		mean	RSD		mean	RSD		mean	RSD	
sample	µg/plate	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR
NC (DMSO		n.d.			40	8		n.d.			n.d.		
Lumacron Red PGA	0.5	n.d.			35	19	0.9	n.d.			n.d.		
	5	n.d.			36	28	0.9	n.d.			n.d.		
	50	n.d.			78	3	**2.0**	n.d.			n.d.		
	500	n.d.			235	10	**5.9**	n.d.			n.d.		
	5000	n.d.			185	8	**4.7**	n.d.			n.d.		
PC *	*	n.d.			197	2	5.0	n.d.			n.d.		
NC (DMSO		17	42		32	20		125	16		124	11	
Olive Cibanone 2R MD	0.5	25	30	1.5	37	15	1.2	112	6	0.9	115	8	0.9
	5	19	24	1.1	32	21	1.0	108	14	0.9	127	19	1.0
	50	24	18	1.4	40	4	1.3	112	8	0.9	121	9	1.0
	500	25	20	1.5	75	11	**2.4**	124	12	1.0	135	19	1.1
	5000	25	14	1.5	26	2	0.8	149	15	1.2	180	10	1.4
PC *	*	390	11	23.4	395	6	12.5	369	1	3.0	641	11	5.2
NC (DMSO		21	15		33	25		124	7		108	6	
Orange Minerprint 3RL	0.5	15	43	0.7	28	29	0.9	117	3	0.9	102	8	0.9
	5	18	51	0.9	30	9	0.9	117	11	0.9	88	18	0.8
	50	34	17	1.6	32	3	1.0	120	12	1.0	118	9	1.1
	500	115	20	**5.6**	37	29	1.1	134	8	1.1	115	12	1.1
	5000	221	15	**10.7**	137	14	**4.2**	143	4	1.2	121	7	1.1
PC *	*	352	7	17.0	73	7	2.2	336	14	2.7	544	10	5.0
NC (DMSO		31	14		35	11		125	10		105	11	
Rouge Imperon K-B	0.5	25	10	0.8	35	15	1.0	108	14	0.9	98	8	0.9
	5	26	22	0.8	30	5	0.9	102	9	0.8	80	15	0.8
	50	29	18	0.9	31	27	0.9	98	11	0.8	98	15	0.9
	500	38	21	1.2	41	17	1.2	107	6	0.9	118	23	1.1
	5000	76	8	**2.5**	315	7	**9.0**	87	15	0.7	180	16	1.7
PC *	*	481	7	15.7	202	8	5.8	668	13	5.3	425	1	4.1

NC: Negative Control ($H_2O_{deion.}$)

PC: Positive Control (TA98-: Nitrofluorene 1.5 µg/plate;
TA98+: 2-Aminoathracene 2.0 µg/plate;
TA100-: Sodiumazide 0.5 µg/plate;
TA100+: 2-Aminoanthracene 2.0 µg/plate)

IR: Induction Rate
(number of revertants sample / number of revertants control)

RSD: relative standard deviation of mean [%]

		TA98 without S9			TA98 witht S9			TA100 without S9			TA100 with S9		
	concentration	mean	RSD		mean	RSD		mean	RSD		mean	RSD	
sample	µg/plate	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR	n=3	[%]	IR
NC (DMSO		21	7		n.d.			108	4		n.d.		
Rouge Terasil P3G	0.5	20	13	1.0	n.d.			123	5	1.1	n.d.		
	5	23	18	1.1	n.d.			112	20	1.0	n.d.		
	50	68	23	**3.3**	n.d.			129	5	1.2	n.d.		
	500	313	15	**15.1**	n.d.			135	5	1.3	n.d.		
	5000	1605	5	**77.7**	n.d.			268	13	**2.5**	n.d.		
PC *	*	355	17	17.2	n.d.			458	3	4.3	n.d.		
NC (DMSO		37	27		45	18		109	8		99	9	
Sirius Grau K-CGL	0.5	27	26	0.7	48	13	1.1	99	11	0.9	93	8	0.9
	5	34	15	0.9	47	6	1.1	110	16	1.0	81	28	0.8
	50	41	7	1.1	47	11	1.0	104	3	1.0	86	2	0.9
	500	123	6	**3.3**	50	8	1.1	100	3	0.9	107	11	1.1
	5000	223	1	**6.0**	108	4	**2.4**	103	11	0.9	95	5	1.0
PC *	*	330	14	8.9	323	11	7.2	408	26	3.7	482	10	4.9
NC (DMSO		24	33		32	10		150	15		124	11	
Turquoise Cibacrone P-GR Liq. 50 %	0.5	19	34	0.8	31	10	1.0	129	8	0.9	121	18	1.0
	5	18	12	0.8	35	13	1.1	137	5	0.9	123	18	1.0
	50	17	27	0.7	32	15	1.0	141	12	0.9	128	13	1.0
	500	21	46	0.9	31	10	1.0	145	13	1.0	117	11	0.9
	5000	43	8	1.8	84	3	**2.6**	140	6	0.9	127	5	1.0
PC *	*	340	19	14.2	340	15	10.5	371	5	2.5	719	14	5.8

NC: Negative Control ($H_2O_{deion.}$)

PC: Positive Control (TA98-: Nitrofluorene 1.5 µg/plate;
TA98+: 2-Aminoathracene 2.0 µg/plate;
TA100-: Sodiumazide 0.5 µg/plate;
TA100+: 2-Aminoanthracene 2.0 µg/plate)

IR: Induction Rate
(number of revertants sample / number of revertants control)

RSD: relative standard deviation of mean [%]

Table 3: *List of dye products tested in the mouse lymphoma assay (MLA). Positive products are marked with grey and the highest induction rates obtained are given*

Dye Product Name	**without S9**	**with S9**
Astrazon Blue BG 200% 01	positive IR 7.4 with 40 µg/ml	n.t.
Astrazon Blue FGRL 200%	positive IR 2.2 with 50 µg/ml	n.t.
Bemaplex Black C-2B	positive IR 4.1 with 313 µg/ml	n.t.
Bleu Terasil 3R-02	positive IR 15.2 with 2,500 µg/ml	n.t.
Brun Cibanone BR MD liq. 40%	negative	negative
Erionyl Bordeaux A-5B	negative	positive IR 9.5 with 625 µg/ml
Olive Cibanone 2R MD	negative	negative IR 2.0 with 5,000 µg/ml
Rouge Imperon K-B	contradictory IR 2.3 with 1,581 µg/ml	negative
Turquoise Cibacrone PGR liq. 50%	negative	positive IR 3.0 with 5,000 µg/ml

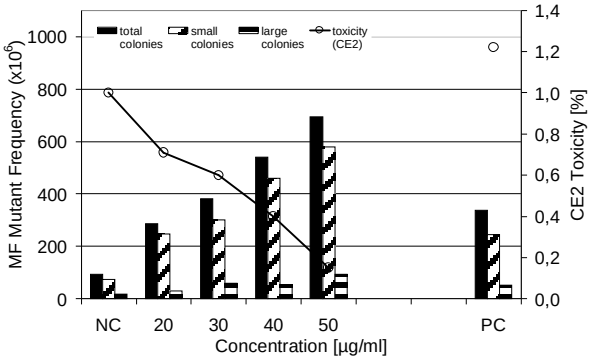
n.t. not tested; IR Induction Rate (mutant frequency sample/mutant frequency control)

4. Discussion

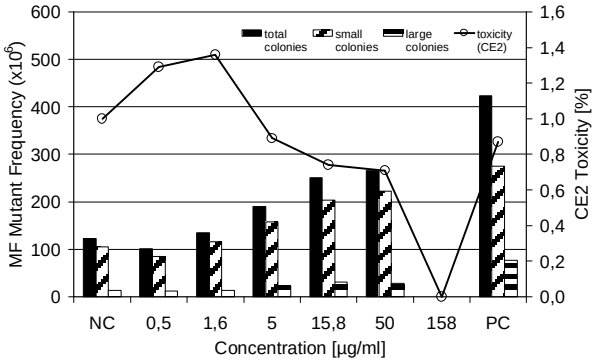
Most of the textile dyes on the market belong to the so-called "existing substances". Mutagenicity data for these substances are often scarce. Several of the textile dye products tested for reverse mutations in *S. typhimurium* and in the mouse lymphoma assay proved to be mutagenic in both assays. These tests fulfill the criteria for the base-level testing requirements of the EU Technical Guidance Document (TGD) [10]. According to the testing strategy of the EU TGD positive results in both tests would warrant additional testing *in vivo* or reconsideration of the marketing of the compound. Project budget did not allow testing in the MLA for all dyes which responded in the bacterial reverse mutation assay. Therefore dye products, which were important for the TFCs due to the use of high volume were selected.

Figure 1: *Dose-response results of mouse lymphoma assay (MLA)*

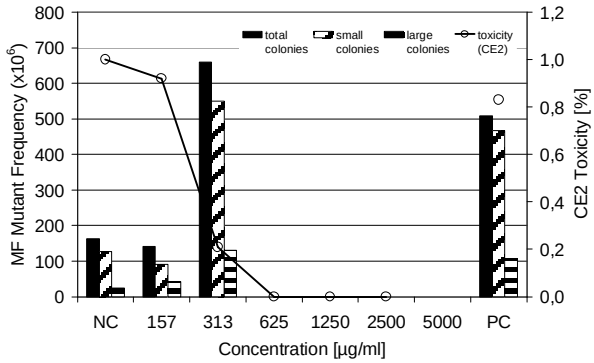
Astrazon Blue BG 200% 0.1 (without S9)



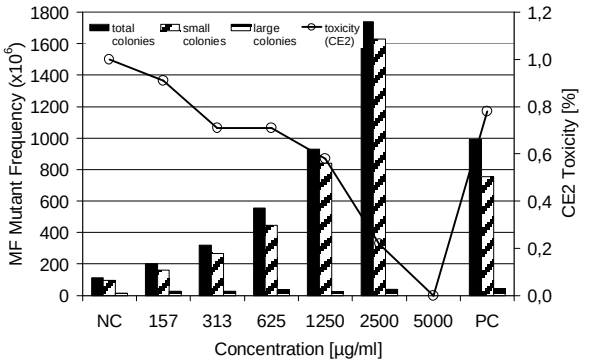
Astrazon Blue FGRL (without S9)



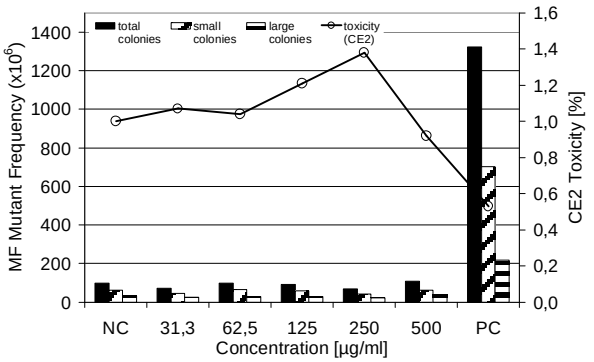
Bemaplex Schwarz C-2B (without S9)



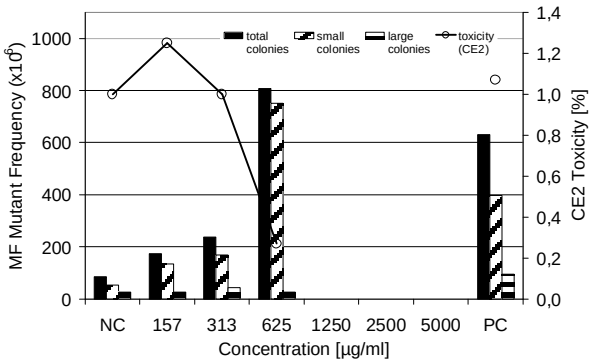
Bleu Terasil 3R-02 (without S9)



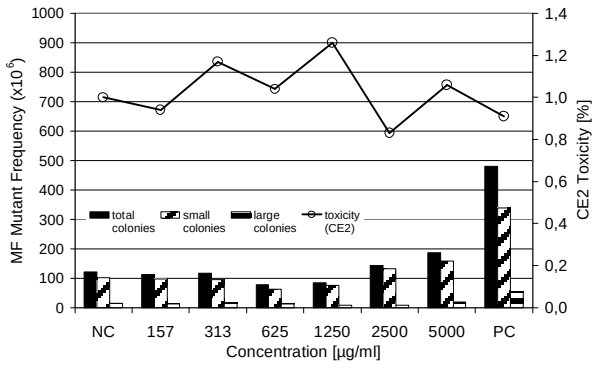
Erionyl Bordeaux A-5B (without S9)



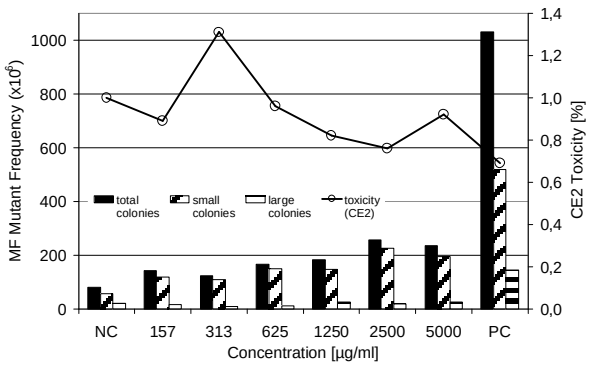
Erionyl Bordeaux A-5B (with S9)



Turquoise Cibacrone PGR liq. 50% (without S9)



Turquoise Cibacrone PGR liq. 50% (with S9)



NC: negative control;
PC positive control (without S9: 4-Nitroquinolineoxide 190 µg/ml, with S9: Benzo-a-pyrene 4 µg/ml)

The MLA makes it possible to distinguish large and small colonies. It is thought that small colonies are induced by chromosome damage and the large colonies by gene mutations [18, 19]. Accordingly it would be expected that Ames positive samples should be characterised by an induction of large colonies in the MLA. However such an effect could not be detected in most samples analysed here. Comparing the IRs for the mutation frequencies of small colonies with those for large colonies did not reveal any substantial differences. Only with Bemaplex Black, mutant frequency was higher with large colonies than with small colonies which coincide with gene mutation effects. Erionyl Bordeaux and Turquoise Cibacrone showed no increased mutation frequencies with large colonies at all, so it can be assumed that genotoxic effects demonstrated in MLA are primarily due to chromosome mutations. Also the Ames positive samples which were MLA negative showed no higher mutation frequencies when only the large colonies were considered. In summary, no clear correlations between point mutations in the bacterial reverse mutation assay and induction of large colonies in the MLA was found. Clements [20] already noted that colony size cannot necessarily be used to predict whether a chemical substance is a point mutagen or a clastogen.

If the results obtained are taken to be representative of all dye products, then nearly 20% of untested textile dyes may be of concern. Within this EU project 281 textile dye products were checked for availability of data on mutagenic effects. Based on data from the literature, from dye producers and on own laboratory results altogether 14 dye products were judged to show mutagenic activity, 16 dye products were suspicious of being mutagenic due to positive results from one test and for 71 dye products no test results were available at all [21]. From the results shown here it can be assumed that within the 71 untested dye products about 14 would show positive results in vitro. Taken together, for all 281 textile dye products the percentage of possibly mutagenic dyes therefore is estimated to be higher than 10%.

In the past various research groups used the Ames and other bacterial tests, as well as mammalian cell test systems and *in vivo* assays (e.g. induction of micronuclei in bone marrow cells) to investigate possible mutagenic effects of dyes used for textile finishing [22 – 27]. Most of these investigations focussed on anthraquinone and azo dyes. Comparisons with our results are difficult as we tested ready-to-sell products, which may consist of mixtures of dyes and additionally contain auxiliaries. However the observations made by several investigators that a significant proportion of dyes cause mutagenic effects in various test systems is corroborated by our findings. Friedman et al. [28] and MacGregor et al. [29] investigated formulated textile dye products in the bacterial reverse mutation assay. Their respective findings of 32 and 29% dyes positive in *S. typhimurium* are in good agreement with our results. These and other published data on single dye ingredients or dye products were used to evaluate other dye products used by textile finishing companies within this EU research project [21].

The dyes discussed here were chosen for testing because no mutagenicity data had been published previously on them. As far as chemical nature of the dyes is revealed in the safety data sheets, no specific chemical group emerges as especially important. Bleu Terasil 3R-02 contains Disperse Blue 999 (Cas-No. 1594-08-7), an anthraquinone dye, but also azo dyes. Both Astrazon Blue FGRL and Astrazon Blue BG comprise Basic Blue 3 (Cas-No. 73570-52-2), which is based on a phenoxazine structure. Astrazon Blue BG contains in addition another basic dye, Basic Blue 159. Bemaplex Schwarz C-2B contains mainly Acid Black 107 (Cas-No. 12218-96-1), a metal complex dye of unknown structure. With Turquoise Cibacrone PGR the safety data sheet only states that the dye belongs to the phthalocyanine dyes; no indication of chemical nature is given for Erionyl Bordeaux A-5B. Olive Ci-

banone 2RMD and Brun Cibanone BRMD, which gave negative results in the MLA, consists of anthraquinone dyes, whereas Rouge Imperon K-B, which also proved to be negative in the MLA, contains Pigment Red 146 (Cas.-No. 5280-68-2), an azo dye. In some cases, impurities may also be influencing the test outcome. In the case of Turquoise Cibacrone PGR different results were obtained in the bacterial reverse mutation assay when the formulated product and the technical grade, pure dye were tested. These data are reported elsewhere [21]. In this situation tracing back mutagenicity to single components is difficult. Nevertheless the formulated products are the material which workers and possibly consumers via textiles are exposed to.

With regard to consumers, exposure to dangerous substances may depend on the fastness of the dyes used. Whereas many of the textile dyes, especially those of high quality, show high fastness, at least some dyes may be leachable from fabrics. Knasmüller et al. [2] obtained positive results with 18 textile samples (9.2%) when they exposed *S. typhimurium* directly by placing pieces of fabric on the agar plates and looking for reverse mutations in the so called "Ames spot test". Within this project similar results were obtained. Five percent of textile samples tested showed positive responses in this test (for details see [30]), supporting the observations of Knasmüller et al. [2] and indicating bioavailability of the dyes. In response to the obtained results, the textile finishing companies participating in the EU-project stopped the use of dyes which proved to be mutagenic in mammalian cells and substituted less harmful substances for them.

Acknowledgments

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