

Detection of Mutagens in Leather and Tannery Industry Assay of 28 Commercial Mixtures

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28 commercial mixtures compounds used in the leather and tannery industries of the "Comprensorio del Cuoio" (leather industry area near Florence) were tested using the Salmonella-mammalian mycosomes mutagenicity test in order to evaluate possible environmental genetic hazard. 5 samples were found to be mutagenic; of them two are involved in the tanning process, one in the liming and the remaining two in the dyeing of leather.

It is well known that tannery workers are potentially exposed to various carcinogens (Buiatti et al., 1980; IARC 1981). To date studies concentrated on the investigation of pure compounds with known chemical concentration. However, substances which tannery workers are exposed to are complex mixtures containing a wide variety of compounds and are potentially capable of causing additive, antagonistic and synergistic biological effects. Using the sensitive short-term Ames test we tried to isolate and identify the biohazardous substances to be submitted to further more complex analysis (Ames et al., 1975).

MATERIALS AND METHODS

The 28 tested products were collected directly from the industries. They are of very common use and representative of the processing phases of disinfection, liming, tanning and dyeing. They are indicated by the commercial names (Tab.1); the composition - as reported by the producers - is given only for the positive products (Tab.2). The presence of Cr(VI) was checked using the S-diphenyl carbazide reagent (Ege et al., 1974).

Ames Salmonella typhimurium tester strains TA 100, TA 98, TA 1537 and TA 1535 were used to determine the mutagenic activity.

The S9 fraction of livers of phenobarbital, β -naphthoflavone pretreated mice was used as activating system. Protein concentration and aminopyrine-N-demethylase activity were determined by the methods of Lowry and of Mazel respectively, in order to test the effectiveness of the

Table 1. Commercial names and category of tested compounds

Tannings	Dyes	Disinfectants	Liming agents	Oils, Resins, Enzymes
Chromitan B	Nero Paraderma	Fungitol S	Erhavit A	Super Oil
Chromitan MS	Bruno Avival RP/K3	Preventol L		Levertan F
Silican	Rosso Avival RP/K3			Betamina
Synthetic Tannin Neosin SSA	Rosso HR			Uroplen DM
Synthetic Tannin Neosin PFB	Gelber Ocra			Uroplen A
Synthetic Tannin Blancotan PRV	Giallo Anilina			Prefondo RL
Estratti di casta <u>g</u> no italiano				Sinalbil
Riconcia HI				Clartan
Tanigan Pak				Lucidol S
Chromosolve				

Table 2. Composition of the 5 positive products as reported by the producers. Composition of Rosso Avival and Bruno Avival is given by the numbers of color index

Chromitan MS	Chelate Basic Chromium Sulphate Cr ₂ O 30% Basicity 50%
Chromitan B	Basic Chromium Sulphate Cr ₂ O ₃ 26% Basicity 32-35%
Rosso Avival	Acid Red 22
Bruno Avival	Acid Yellow 118 Acid Red 227 Acid Brown 56
Erhavit A	Dimethylamine Urea Thioglycolic Acid H ₂ O

fractions used (Lowry et al., 1951; Mazel, 1971). The mice (Swiss albino CD₁ strain), maintained at room temperature with ad libitum access to a standard diet and tap water, received Na-phenobarbital (100 mg/kg) i.p. on the first and the third day, Na-phenobarbital (50 mg/kg) and β -naphthoflavone (80 mg/kg) on the second day and were sacrificed on the fourth day.

The products were tested according to the standard method described by Ames & coll.; at least three tenfold dilutions for each product were plated starting with 0,1 ml of the pure product for the liquids and 0,1 ml of the saturated solution for the powders. The samples were dissolved in distilled water or in dimethyl sulfoxide.

RESULTS AND DISCUSSION

Name and usage of the 28 tested products are given in Tab. 1; five of them resulted positive on one or more strains. They induced a more than twofold increase in the number of his⁺ revertants per plate with respect to the controls and a quite linear dose-response (Tab.3). Chromitan B and Chromitan MS are involved in the tanning phase of leather processing (Bronzetti et al., 1983). Both contained Chromium (III) but no Chromium (VI) was

Table 3. Every value is the mean of the results of 4 experiments. Three different concentrations were tested with and without metabolic activation.

COMPOUND	CONC/PLATE	TA 100		TA 98		TA 1537		TA 1535	
CHROMITAN B	50 mg	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
		tox	tox	tox	tox	tox	tox	tox	tox
		60	300	tox	49	tox	tox	80	192
CHROMITAN MS	4 mg	120	237	20	47	tox	16	14	44
		50 mg	60	tox	11	tox	tox	tox	tox
		10 mg	127	225	19	tox	26	78	182
ROSSO AVIVAL	4 mg	95	158	27	50	11	10	37	43
		0.1 ml	326	454	505	177	180	39	25
		10 λ	193	419	469	110	113	26	24
BRUNO AVIVAL	1 λ	142	166	199	438	27	21	17	23
		0.1 ml	210	253	307	123	121	19	29
		10 λ	141	202	200	73	59	9	26
ERHAVIT A	1 λ	133	164	122	103	19	43	8	19
		0.1 ml	135	tox	tox	tox	tox	tox	101
		10 λ	178	41	72	10	17	55	55
CONTROL	1 λ	186	118	36	58	15	12	32	22
		122	139	40	41	11	12	16	19

detected. It is well known that Chromium (VI) is both mutagenic and carcinogenic, while in some cases Chromium (III) was found to be genetically active only when in the form of a stable complex with organic compounds such as aromatic amines. Rosso Avival and Bruno Avival showed a considerable mutagenic effect on the strains TA 98 and TA 1537 both in presence and in absence of metabolic activation suggesting the occurrence in both products of a direct acting frameshift mutagen. A very weak effect was observed also in the strain TA 100 but it was not regarded as significant. Erhavit A, Chromitan B and MS resulted positive principally on the strain TA 1535 with and without metabolic activation.

None of the disinfectants tested showed mutagenic activity on the Salmonella tester strains but, due to their high toxicity for bacteria, they had to be assayed at very low concentrations.

Our results suggest that, in order to evaluate the environmental health risk,, research should not be limited to the study of well-known active compounds, but should also analyse complex mixtures as they occur in the environment. Rosso Avival and Bruno Avival are employed in the dyeing process, Chromitan B and MS in the tanning and Erhavit A in the liming suggesting that it is quite impossible to single out a particular risky phase in the whole industrial process.

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