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Detection of Genotoxicants in the Leather and Tannery Industry Using Short-term Tests

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One of the most pressing problems for mankind is the determination and evaluation of the genetic and carcinogenic risks of environmental agents. Until now studies have concentrated on the investigation of pure compounds or agents with known chemical concentrations. However, studies of the complex mixtures employed in the work place are considerably more difficult. Such mixtures contain a wide variety of compounds at different concentrations and are potentially capable of causing additive, antagonistic, or synergistic responses in test organisms. For this reason such complex environmental contaminations are not studied, and consequently there does not exist a large literature on the subject.

The problems of environmental hazard assessment are very complex and difficult, but at the same time they are of fundamental importance. To obtain useful results, it is necessary to carry out an interdisciplinary program involving the integration of analytical chemical and biological investigations.

The hide and leather industry is of considerable importance in Italy with special concentration in the region of Veneto, Campania, and Tuscany. In Tuscany, Tanneries are concentrated near Florence in the "Comprensorio del Cuoio" (Leather Industrial area) in which there are six small municipalities whose inhabitants numbered nearly 90,000 in the 1980's.

The population in this area is, on the average, younger than the overall Italian population because of the large number of immigrants of working age and a higher natality index, and it is probably increasing.

The majority of the working population is employed in the leather industries, particularly in the tanning industry (69% of the total number of employees). However, the problems of the tannery industry are not restricted to the region of Tuscany, but exist wherever hide and leather are manufactured.

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This investigation is based on an interdisciplinary research program involving the construction of risk maps delimiting those zones of increased tumor incidence as determined by epidemiologists. From these zones of high tumor incidence, samples of prospective carcinogenic agents were obtained. Qualitative analyses of the compounds were conducted, and extracts were prepared in order to study the specific genetic activity "in vitro" and "in vivo" using appropriate strains of microorganisms. At the same time, we examined the enzymatic alteration in liver fraction brought about by the agents being tested.

This paper reports the results obtained from short-term tests using diploid strain D7 of Saccharomyces cerevisiae and Salmonella typhimurium strains. Two tanning agents containing chromium three, Chromitan B and Chromitan MS , which are widely used in this type of industry were investigated.

MATERIALS AND METHODS

Yeast strain

Saccharomyces cerevisiae strain D7 was obtained from Dr. F.K. Zimmermann. The diploid strain D7 can detect, simultaneously, mitotic gene conversion at the trp5 locus, point (reverse and suppressor) mutation of the mutant allele, ilv-92,and mitotic recombination between the centromere and the ade2 locus. Mitotic crossing over can be detected visually as pink and red twin-sectored colonies which are due to the formation of homozygous cells of the genotypes ade2-40/ade2-40 (deep red) and ade2-119/ade2-119 (pink) from the originally heteroallelic condition ade2-40/ade2-119 which forms white colonies. Mitotic gene conversion can be detected by the appearance of tryptophan-nonrequiring colonies on selective media. The alleles involved are trp5-12 and trp5-27 derived from the widely used strain D4. Mutation induction can be followed by the appearance of isoleucine-non-requiring colonies on selective media. D7 is homoallelic ilv1-92/ilv1-92 (ZIMMERMANN et al.1975). In this work we consider mitotic gene conversion and reverse point mutation, which are sufficient to give indications of genetic effects (BRONZETTI et al.1978,1981).

Mutagenic activity was determined using Salmonella typhimurium strains TA 100, TA 1538 and TA 1535 with and without metabolic activation as described by Ames and Co-Workers (AMES et al.1975). Dose response curves were obtained by plate test at different concentration of compounds dissolved in water; data points given are the mean of three different experiments.

Metabolizing enzymes

Protein concentration and aminopyrine-N-demethylase activity were determined, the first by the method of MAZEL (1971) and the other by the procedure of LOWRY (1966) in hepatic fractions of phenobarbital β-Naphthoflavone pretreated mice. Mice received repeated administrations of Chromitan B and Chromitan MS (50 mg/Kg daily for 4 days for a total of 200 mg/Kg).

In the hepatic fraction the protein content was determined by the Lowry method using bovine serum albumin as standard.

Mice

Experimental animals employed in the preparation of the S9 hepatic fraction were Swiss albino CD1 strain. They were maintained at room temperature with ad libitum access to a standard diet and tap water. They received Na-phenobarbital (100 mg/kg) i.p. the first day, phenobarbital (50 mg/kg and β-Naphthoflavone (80 mg/kg) the second day and Na-phenobarbital (100 mg/kg) the third day. On the fourth day, they were killed.

Chemicals

Chromitan B and Chromitan MS were purchased from BASF Aktiengesellshaft. Chromitan B contained 26% by weight Cr_2O_3 in basic condition. In our analysis all chromium present was in the form of chromium three. No chromium six could be detected.

Suspension test procedure

For experiments using Salmonella strain the Ames procedure was used (AMES et al.1975) and for those involving yeast strain D7 the protocol of Zimmermann and Bronzetti (ZIMMERMANN et al.1973; BRONZETTI et al.1978, 1981) was employed.

RESULTS AND DISCUSSION

Chromitan B and Chromitan MS induced point mutation in Salmonella typhimurium strain TA 100, TA 1538 and TA 1535 with activated metabolism system (S9). Without liver microsomal fraction the agents are quite toxic but they did not induce detectable genetic effects (Tables 1-2). In plate tests TA 1538 and TA 100 are more sensitive than TA 1535 both with Chromitan B and Chromitan MS. Chromitan B is more active that Chromitan MS. The concentrationsused were 50 and 100 mg/pla-

te and, as can be seen in Table 1, there is a strong positive correlation between dose and effect. In yeast strain D7 both Chromitan B and Chromitan MS showed high levels of toxicity. However genetic effects in this same strain were not considered (Fig.1). From analytical studies in our laboratory as reported above, these industrial compounds were found to contain chromium three; by contrast chromium six was sought for but was not detected.

It is well known that chromium six is both mutagenic and carcinogenic while studies involving chromium three have resulted in conflicting results.

Investigations are in progress to determine if the mutagenicity and toxicity found in our studies depend upon chromium three itself, or the total molecular structure of which it is a part, or the complex industrial chromium mixtures.

Table 1. Every value is the mean of the results of 4 experiments

Number of revertants/plate of some strain of S.typhimurium after treatment with Chromitan B

	TA 100		TA 98		TA 1538		TA 1535	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
50mg/p	tox	tox	tox	tox	tox	tox	tox	tox
10mg/p	60	300	tox	49	16	138	80	192
4mg/p	120	237	20	47	15	37	14	44
Control	113	146	25	40	32	46	24	51

Table 2. Every value is the mean of the result of 4 experiments

Number of revertants/plate of some strains of S.typhimurium after treatment with Chromitan MS

	TA 100		TA 98		TA 1538		TA 1535	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
50mg/p	tox	60	tox	11	tox	16	19	25
10mg/p	127	225	19	39	23	41	78	182
4mg/p	95	158	27	50	39	47	37	43
Control	113	146	25	40	32	46	24	51

Table 3. Aminopyrine demethylase activity in mice liver after induction and repeated administrations of 50 mg/kg chromitan B and chromitan MS

	Aminopyrine demethylase µmol CH_2O/h x g liver	Proteins mg/ml S9
Chromitan B	5.12±0.87	9.5±0.21
Chromitan MS	10.3±1.02	11±0.37
Control	23.41±0.52	21.5±0.50

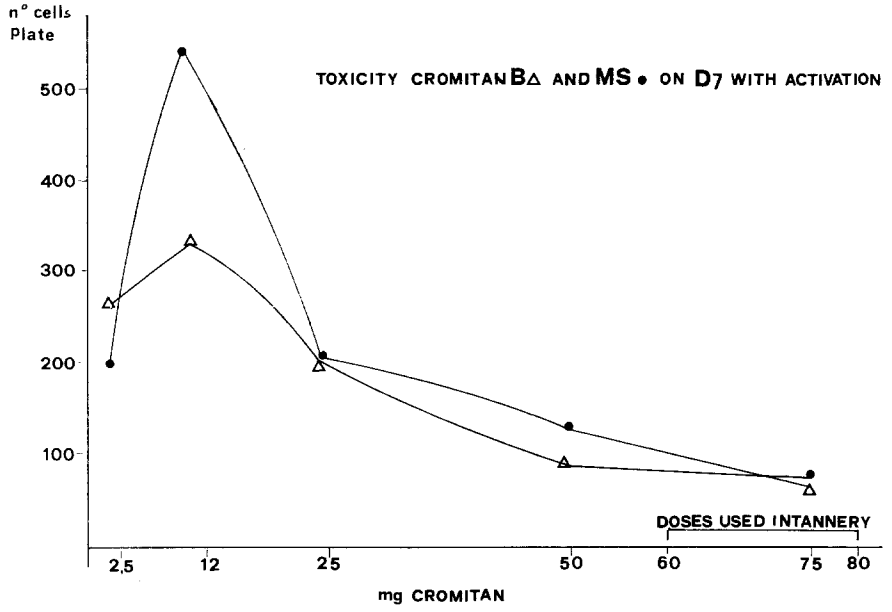

Figure 1. Toxicity of chromitan B and MS in D7 strain of S.cerevisiae with activating system

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