

## Genotoxic exposure of workers creosoting wood

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**ABSTRACT** In a small wood preserving industry spot samples were taken from contaminated surfaces at several places and tested for mutagenicity. The results suggest that the application of a wipe test can give a first indication of occupational exposure to mutagenic and carcinogenic substances, particularly when exposure occurs more from skin contact than from inhalation. One of the pesticide chemicals used to preserve wood is the mutagenic creosote. It was found that mutagens appeared in the urine of rats after intraperitoneal administration of creosote. Despite these results, no increase in mutagenicity could be detected in the urine of creosote workers in relation to their work.

Creosote is well known as one of the pesticide chemicals used for wood preservation. It is a mixture of oils that are separated in the distillation of coal tar and consists principally of liquid and solid hydrocarbons. Because coal tar is known to contain many polycyclic aromatic hydrocarbons, we were interested to know if creosote was mutagenic and showed it to be so with the Salmonella/microsome assay.<sup>1</sup>

Because of its mutagenicity this substance might become a potential hazard to workers engaged in the preservative treatment of wood. Production workers in the wood preserving industry can be exposed to creosote not only by the inhalation of vapours but also through skin and eye contact. It is known that workers exposed to coal tar products have a high risk of cancer.<sup>2</sup>

In the present study the possibilities of detecting exposure to creosote were explored. At several places in the work area contaminated surfaces were examined for the presence of mutagens. In addition, since it was found that exposure of Wistar rats to creosote under experimental conditions leads to the excretion of mutagenic products in urine, an attempt was made to monitor creosote workers by measuring urinary mutagenicity.

### Material and methods

#### CHEMICALS

The creosote type P1 that was used was manufactured by Cindu Chemicals BV (Uithoorn, The

Netherlands). Bacterial  $\beta$ -glucuronidase (type IX) was obtained from Sigma (St Louis, USA), Amberlite, type XAD-2 from Serva (Heidelberg FRG), purified agar from Difco Laboratories (Detroit, USA), and nutrient broth No 2 from Oxoid Ltd (Basingstoke, England). All other chemicals used were of the highest purity obtainable.

#### IMPREGNATION PROCESS

The impregnation of wood with creosote is accomplished by a pressure method (full cell process). After a charge of wood is taken into a cylinder, an initial vacuum is applied for a period of at least 15 minutes. The vessel is then filled with creosote having a temperature of at least 70°C (mostly 85–100°C), still maintaining the vacuum. Next, the vacuum is released and pressure with a maximum of 10 kgf/cm<sup>2</sup> is applied to the system. Pressure is maintained until the required gross absorption of creosote has been achieved (maximal three hours). This value varies depending on the species being treated. At the end of the pressure cycle, the pressure is reduced to atmospheric level, the preservative returned to storage, and the treated wood often subjected to a final vacuum to remove excess creosote oil from the surface of the stock. The vacuum is released, the door of the vessel opened, and the treated wood removed.

#### WORKERS

We have studied the possible exposure of three workers. One operates the cylinder and the other two move the wood in and out of the cylinder. The operator especially would be briefly exposed to

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creosote vapour when the cylinder door is opened after treatment. The workers may also be exposed to residual surface creosote through skin contact, although most of the time they wear gloves.

#### MUTAGENICITY TESTING

The mutagenicity test was performed according to Ames *et al.*<sup>3</sup> with *Salmonella typhimurium* TA98 and TA100. We used Oxoid nutrient broth instead of Difco nutrient broth. Rat liver S9 (9000 g supernatant) fractions were prepared from male Wistar rats, pretreated with aroclor 1254; S9 mix contained 0.1 ml S9 per ml. Every determination was done in triplicate. After incubating the plates for 48 h at 37°C the number of revertant colonies was counted.

#### URINARY MUTAGENICITY

##### Rats

Male Wistar rats weighing about 200 g were purchased from TNO (Rijswijk, The Netherlands). The animals were housed individually in stainless steel metabolic cages, designed for the separate collection of urine and faeces. The rats had free access to water and food (Hope Farms, Woerden, The Netherlands). Creosote, dissolved in olive oil, was injected intraperitoneally; control rats received injections of olive oil only.

Urine samples were collected for 24 h and stored at -20°C until assayed. Before the assay individual samples were made up to 15 ml and sterilised by filtration through 0.2 µm membrane filters.

Mutagenicity of the urine samples was determined using the *S typhimurium* strains TA98 and TA100: 0.1 ml of a full grown suspension of the bacteria ( $\pm 2 \times 10^9$  bact/ml) was added to the topagar containing 0.3 ml of diluted urine, and was supplied with an activating enzyme system. This activating enzyme system consisted of either 0.5 ml S9 mix or 0.1 ml of a sterile  $\beta$ -glucuronidase solution (1500 U/ml), or a combination of both.<sup>4</sup>

##### Workers

Urine samples from the workers were gathered during 10 consecutive days, including two free weekends. During this period two urine samples were collected daily: one overnight and one between 10 am and 4 pm. The mutagenicity of the urine was determined according to van Doorn *et al.*<sup>5</sup>

#### MONITORING OF CONTAMINATED SURFACES

To detect contamination of work areas with mutagenic creosote we applied a method based on that described by Simmon and Peirce.<sup>6</sup> Five millilitres of acetone or alcohol were put on a surface and the solvent was mopped up with a Kleenex tissue. The tissue was extracted twice with 25 ml of acetone

or alcohol. The acetone or alcohol was evaporated to dryness using a rotary evaporator. The total residue was dissolved in 5 ml of dimethylsulphoxide (DMSO), and 0.1 ml of this solution was added per plate and assayed for mutagenicity with the *S typhimurium* tester strain TA98 in the presence of S9 mix. Every determination was done in triplicate. After 48 hours' incubation at 37°C the number of revertant colonies on the plates was counted.

#### Results

##### MUTAGENICITY OF CREOSOTE

Table 1 shows the experimental results in which the mutagenicity of creosote was established. The mutagenicity was assayed using the *S typhimurium* strains TA98 and TA100 in the presence of S9 mix and expressed as the number of revertant colonies per plate.

Table 1 *Mutagenicity of creosote*

Creosote (µg/plate)	No of his <sup>+</sup> revertants per plate*	
	Strain	
	TA98	TA100
0	16 ± 4	69 ± 4
2	17 ± 1	87 ± 9
5	21 ± 2	99 ± 2
20	134 ± 9	284 ± 12
50	336 ± 7	598 ± 9

\*Mean values ± SEM of determinations in triplicate.

##### DETECTION OF MUTAGENIC SUBSTANCES IN THE WORK ENVIRONMENT

The work environment was examined for the presence of mutagenic substances. Spot samples were taken at several places from contaminated surfaces and tested for mutagenicity; the results are shown in table 2. Spots Nos 1, 2, 3, and 4 were on metal

Table 2 *Contamination with mutagenic substances of some areas of the work environment\**

Sample	No of his <sup>+</sup> revertants per plate†	
	Solvent used for cleaning	
	Acetone	Alcohol
Spot 1	510 ± 29	395 ± 8
Spot 2	558 ± 52	77 ± 2
Spot 3	435 ± 31	109 ± 5
Spot 4	231 ± 7	373 ± 20
Spot 5	181 ± 9	23 ± 1
Negative control	45 ± 3	31 ± 2
Positive control	‡	324 ± 8
Spontaneous	19 ± 2	

\*Experimental details were as mentioned in materials and methods.

†Mean values (± SEM) of three determinations on the same sample.

‡No value available because of a toxic effect on the bacteria.

Table 3 Mutagenicity towards the *Salmonella typhimurium* strains TA98 and TA100 of urine from a creosote treated rat after the addition of different enzyme preparations

Addition‡	No of his <sup>+</sup> revertants/plate*			
	Treatment			
	Creosote†		Control	
	TA98	TA100	TA98	TA100
None	43 ± 2	188 ± 9	37 ± 1	175 ± 8
S9 mix	44 ± 5	202 ± 8	39 ± 4	151 ± 3
S9 mix +				
β-glucuronidase	123 ± 8	341 ± 10	43 ± 3	175 ± 12
β-glucuronidase	76 ± 11	275 ± 18	47 ± 2	166 ± 2

\*Mean values (± SEM) of three measurements of the same urine.

†Rats were injected intraperitoneally with a dose of 250 mg/kg.

‡For experimental details see materials and methods.

One hundred and fifty units of β-glucuronidase were used.

surfaces. Spots 1 and 2 were on a grip of the door of the cylinder and on a handle very close to this door, respectively. Spot 3 was on the banisters about 3 m away from the cylinder. Spot 4 was on a lorry used to transport the wood in and out of the cylinder. Spot 5 was on wood that was not treated with creosote and which was piled up about 15 m away from the cylinder. A negative control was made from the surface of a clean table in our laboratory. A spot on the surface of creosoted wood served as a positive control. These results show that the work environment is contaminated with mutagenic substances. Extraction with acetone gave higher mutagenic values than extraction with alcohol.

#### ANIMAL EXPERIMENTS

Administration of creosote to rats (250 mg/kg bodyweight, intraperitoneally) resulted in the appearance of mutagens in the urine detectable with the strains TA98 and TA100 (table 3). The highest mutagenicity values were detected after the addition of S9 mix in the presence of β-glucuronidase to the urine.

#### ABSENCE OF MUTAGENICITY IN URINE OF CREOSOTE WORKERS

We were interested whether the workers engaged in the preservative treatment of wood with creosote had mutagenic urine. We collected urine samples on 10 consecutive days but although using *S typhimurium* TA98 in the presence of S9 mix and β-glucuronidase, we failed to detect an increase in urinary mutagenicity that could be related to their work.

#### Discussion

These results clearly show the presence of mutagenic substances in the work environment of a wood preserving industry, most likely due to the presence of creosote. The method used for

monitoring the work environment for the presence of mutagens is easy to carry out and is based on the method of Simmon and Peirce<sup>6</sup> who introduced it to detect the spillage of carcinogens and mutagens in laboratories. The present results suggest that the application in the work environment of this wipe test could give a first indication of occupational exposure to mutagenic and carcinogenic substances. This might be important, particularly in those cases where exposure can occur through skin contact rather than through inhalation.

Despite the substantial contamination of the work environment with mutagenic substances, no increase in mutagenicity in the workers' urine was detected in relation to their work. On the other hand, mutagens appeared in the urine of rats after intraperitoneal administration of creosote. Most probably the absence of mutagens in the urine of creosote workers can be attributed to (1) a relative low level of exposure—for instance, owing to the wearing of protective gloves, (2) the wrong choice of the time of taking urine samples, and (3) the insensitivity of the urinary mutagenicity assay. Presumably more selective methods of biological monitoring might give more information about internal exposure.

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After completion of this paper we characterised two important mutagenic components of creosote: benzo(a)pyrene and benz(a)anthracene. Concentrations of these components were found to be 0.18% and 1.1%, respectively.<sup>7</sup>

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