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MUTAGENICITY OF POLYURETHANE THERMODEGRADATION PRODUCTS IN THE SALMONELLA/MICROSOME TEST

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ABSTRACT

The oxidative thermodegradation products of non-fireretarded rigid polyurethane foam contain substance(s) which cause both frameshift (TA 98) and base-pair (TA 100) mutations in *Salmonella typhi murium* strains lacking normal excision repair. The mutagenic activity is evident only in the presence of 9 000 × g rat liver supernatant fortified with necessary co-factors. It is also greatly dependent on the temperature of degradation, no mutagenic activity being detected after thermodegradation at 350 °C and high mutagenicity resulting from thermodegradation at 700 °C.

The bacterial test system introduced by Ames and co-workers allows rapid detection of mutagens[1]. Initially the test was applied to single compounds[6]. The application was later extended, e.g. to extracts of particulate matter[3,4,8]. Biological screening with toxicity testing offers an alternative approach that may resolve many difficulties observed in chemical screening. Such a screening test may also detect unidentified mutagenic agents in complex test samples.

Occupational exposure to various degradation products of plastics is predicted to increase dramatically in the future. As a part of our studies on the potential impact of thermodegradation products of polymers on health, the mutagenicity of the aerosol phase components of polyurethane thermodegradation products was evaluated with the *Salmonella*/microsome screening test. The polymer used was degraded in air at several temperatures (350 °C, 500 °C and 700 °C).

MATERIALS AND METHODS

The diphenylmethane-4,4'-diisocyanate (MDI) based polyether polyurethane used in this study was obtained from a company which prepares it for thermal insulation in household devices. It was made from Desmodur® 44V, Desmofen® and the blowing agent Frigen® 11 (Bayer AG, Leverkusen).

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The amount of 150 mg of polyurethane foam was placed in a J-shaped glass tube (inner diameter 7 mm), which was inserted through a hole into an oven heated to 350 °C, 500 °C or 700 °C. To ensure that the temperature of degradation was the same as that of the oven, the shorter arm of the tube was totally in the oven. The J-tube was connected by glass tubing to a glassfibre filter (diameter 37 mm, Sartorius Type 13430). The connections were made by PTFE tubing. Air was drawn through the system at a rate of 0.8 l/min. The time used for degradation was 15 min. In some experiments the filter was replaced by liquid traps containing 2 ml of dimethylsulphoxide. The dimethylsulphoxide solution in the traps was used as such in the Ames test. The filters were macerated in 3 ml of dimethylsulphoxide, the mixture was centrifuged, and the brownish-yellow supernatant was used for the mutagenicity tests.

The mutagenicity test with *Salmonella typhi murium* was performed as described in detail by Ames and co-workers[2]. In this study we strictly followed the Ames procedure. Male rats (200 to 300 g) of the Sprague-Dawley strain were injected with a polychlorinated biphenyl compound, Clophen C (200 mg/kg, obtained from Bayer AG, Leverkusen) in sesame oil 5 days before they were killed. The liver homogenate (S-9) was prepared as described by Ames and co--workers[2]. The tester strains used were TA 98 and TA 100.

In the assessment of the toxicity of the test compounds, 100 μl of a 10^5-fold dilution of the bacterial suspension was plated with the addition of histidine and the test compounds. The survival rate of treated plates is expressed in percentage vs. the number of viable cells of control plates (total number of auxotrophs). The possibility of differential survival between revertants and auxotrophs can never be ruled out without reconstruction experiments.

Calculation was made according to a number of revertants (prototrophs) per 10^5 survivors (auxotrophs). The values given in the tables represent the means of 4–6 estimations of survivors and revertants.

RESULTS

The mutagenicity of the oxidative thermodegradation products of polyurethane on tester strains TA 98 and TA 100 is shown in Table 1. When the product was a result of degradation at 350 °C, no mutagenic activity could be observed in either strain, not even in the presence of 9 000 × g rat liver supernatant fraction fortified with essential co-factors (S-9). However, products of 500 °C and 700 °C degradation exhibited clear mutagenicity in the presence of S-9 mix. A linear dose-response curve of the mutagenicity of the thermodegradation products was obtained in strain TA 98 in the presence of fortified rat liver supernatant fraction (Table 2). Table 2 shows the mutagenicity (revertants/plate + 100 μl S-9/plate) and toxicity (survival rate in percentage vs. controls, bacterial dilution 10^{-5}) in strain TA 98 as well as the results of a reconstruction experiment (revertants/10^5 survivors). Metabolic activation (100 μl S-9/plate) was required for the mutagenic activity to appear. No mutagenicity could be detected if NADP was deleted from the S-9 mix (data not shown). Samples of 100 μl of

TABLE 1
Mutagenicity of thermodegradation products from polyurethane (150 mg) in the *Salmonella*/liver homogenate test.

Degradation product	µl/plate	TA 98 Revertants/plate − S-9mix	TA 98 Revertants/plate + S-9mix	TA 98 Survival rate*	TA 100 Revertants/plate − S-9mix	TA 100 Revertants/plate + S-9mix	TA 100 Survival rate*
PUL-350	50	19	–	–	62	113	
(at 350 °C collected in dimethylsulphoxide traps)	25	31	65	100	101	103	
PUL-500	100	toxic	toxic	4	–	–	
(at 500 °C collected in	50	toxic	464	10	toxic	70	
dimethylsulphoxide	25	toxic	564	40	–	–	
traps)	10	17	255	97	toxic	85	
	5	18	181	100	–	–	
	1	7	41	100	–	–	
PUF-500	100	10	392	74	–	–	
(at 500 °C collected on	50	23	212	89	–	–	
glassfibre filters)	25	33	266	96	–	–	
PUF-700	100	toxic	toxic	1	–	–	
(at 700 °C collected on	50	toxic	832	47	–	–	
glassfibre filters)	25	42	1250	82	53	322	77
	20	33	1300	76	88	319	90
	10	40	477	81	69	174	92
	5	30	296	82	53	123	100
Benzo(a)pyrene	5	35	235		61	532	
	0	31	41		71	76	

*Survival rate in percentage vs. controls, bacterial dilution 10^{-5}.

the thermodecomposition products collected in the dimethylsulphoxide trap were toxic to all strains. Even 50 µl of the same sample was toxic to the bacteria without the addition of S-9 mix. In the presence of liver homogenate the addition of 50 µl of the thermodegradation products showed no toxic effect.

TABLE 2

Mutagenicity and toxicity of thermodegradation products from 150 mg of polyurethane in *Salmonella typhi murium* strain TA 98 in the presence of 9000 × g rat liver supernatant (S-9 mix).

Degradation product	µl/plate	Revertants/plate + S-9 mix	Survival rate*	Revertants/10^5 survivors
PUL-500	100	toxic	4	–
(at 500 °C trapped in	25	564	40	3.06
2 ml of	10	255	97	0.57
dimethylsulphoxide)	5	181	89	0.40
	1	41	100	0.10
	0	41	100	0.09
PUF-500	100	392	74	0.80
(at 500 °C collected on a glassfibre filter)	0	41	100	0.07
PUF-700	25	1250	82	3.8
(at 700 °C collected on	20	1300	76	4.3
a glassfibre filter)	10	477	81	1.5
	5	296	82	0.9
	0	41	100	0.1

*Survival rate in percentage vs. controls, bacterial dilution 10^{-5}.

DISCUSSION

In the past, a considerable amount of data has been obtained on the chemical identity of the thermodegradation products of polyurethane foams[5,7,9]. Both the quality and quantity of the products have been shown to depend greatly upon the temperature of degradation. In these studies, the investigators were primarily interested in the volatile compounds, and data on the aerosol phase (compounds with high boiling points) are scanty.

Polyurethane foams decompose by the elimination of a nitrogen rich material (yellow smoke) at relatively low temperatures (about 300 °C). At higher temperatures (about 800 °C) this smoke is also decomposed[9] along with the polyol component of the foam. In the present study mutagenicity was detected in the aerosol phase. This phenomenon indicates that the component(s) responsible must have relatively high boiling points. It can be speculated that isocyanates react with water vapour and form aromatic amines, which usually have high boiling points. Aromatic amines such as 4,4-methylene-bis(2-chloroaniline) have been shown to be mutagenic in the *Salmonella*/microsome test[6].

The mutagenicity caused by polyurethane thermodegradation products increased as the temperature of degradation increased. The positive response was found to be dependent on the presence of rat post-mitochondrial supernatant in the test system. Metabolic activation is thus required for the mutagenic activity to appear. The exact nature of the mutagenic component(s) remains to be

clarified. The degradation material trapped in dimethylsulphoxide was much more toxic than that collected on glassfibre filters, perhaps because of the presence of hydrogen cyanide. The toxicity was decreased by the addition of S-9 mix, and this finding suggests that the liver homogenate could decrease the toxicity, perhaps through the rhodanase action.

In summary, evidence has been presented that the aerosol fraction of polyurethane thermodegradation products contain substances that cause both frameshift and base-pair mutations in bacterial strains lacking the ability for normal excision repair. The prospect of a significant increase in occupational exposure to plastic thermodegradation products warrants specific identification of these mutagenic substances.

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REFERENCES

1. *Ames, B. N., Durston, W. E., Yamasaki, E. and Lee, F. D.* Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. U.S.A., **70** (1973) 2281–2285.
2. *Ames, B. N., McCann, J. and Yamasaki, E.* Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res., **31** (1975) 347–364.
3. *Commoner, B., Madyastha, P., Bronsdon, A. and Vithayathil, A. J.* Environmental mutagens in urban air particulates. J. Toxicol. Environ. Health, **4** (1978) 59–77.
4. *Dehnen, W., Pitz, N. and Tomingos, R.* The mutagenicity of airborne particulate pollutants. Cancer Lett., **4** (1977) 5–12.
5. *Einhorn, I. N., Ramakrishnan, M. S., Vorhees, K. J. and Mickelson, R. W.* Thermal degradation of model urethane foams – An analytical study – I. Fourth International Cellular Plastics Conference, The Society of Plastics Industry, Montreal, Nov. 15–19, 1976.
6. *McCann, J., Choi, E., Yamasaki, E. and Ames, B. N.* Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A., **72** (1975) 5135–5139.
7. *Murdoch, I. A. and Rigby, L. J.* Thermal volatilization analysis by mass spectrometry. Dyn. Mass Spectrom., **3** (1973) 255–264.
8. *Talcot, R. and Wei, E.* Airborne mutagens bioassayed in Salmonella typhi murium. J. Natl. Cancer Inst., **58** (1977) 449–451.
9. *Woolley, W. D. and Fardell, P. J.* The prediction of combustion products. Fire Res., **1** (1977) 11–21.