

Effects of thermal degradation products of polystyrene on drug biotransformation and tissue glutathione in rat and mouse

by ANTTI ZITTING, L.Sc. (Eng.), PIRKKO PFÄFFLI, M.Sc., and HARRI VAINIO, M.D.¹

ZITTING, A., PFÄFFLI, P. and VAINIO, H. Effects of thermal degradation products of polystyrene on drug biotransformation and tissue glutathione in rat and mouse. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 60—66. Rats and mice were exposed to the fumes of oxidative thermal degradation of polystyrene (350°C). A decrease in the reduced glutathione (GSH) in both liver and lung was detected immediately after both the acute (mice, 3 h) and subacute (rats, 3 weeks) exposures were stopped. Later on an elevation in the amount of GSH due to the increased synthesis (rebound effect) could be seen. Cytochrome P-450 content in mouse liver was initially decreased after acute exposure, but the prolonged treatment doubled the amount of the P-450 hemoprotein in liver microsomes. After acute exposure 7-ethoxycoumarin O-deethylase activity in mouse liver was doubled in 24 h. When the exposures were continued, this enhancement in ethoxycoumarin O-deethylase activity gradually disappeared. O-deethylase activity was also enhanced in rat liver and lung after subacute exposure. The exposures given had no effect on diphenyloxazole hydroxylase, and the effects on the conjugating enzymes (epoxide hydratase, UDPglucuronosyltransferase, glutathione S-transferase) were insignificant in rat liver.

Key words: drug biotransformation, glutathione, polystyrene, thermal degradation.

Polystyrenes are the third most produced type of plastics, after polyethylenes and polyvinyl chlorides (8). As polystyrenes are processed to their final form, high temperatures are used, and thus a possibility exists for the thermal degradation of the polymer and its various additives. The primary degradation products can further react with oxygen or interreact to produce a number of different compounds that can then be emitted to the ambient air. Although the extent of deg-

radation is small at the temperatures used in processing, during the workday enormous quantities of plastics are processed with efficient production machinery so that there is a possibility that remarkable concentrations of decomposition products are present in work areas.

So far, interest in the toxic properties of thermal degradation products has mainly focused on the field of combustion toxicology and acute effects. In this study we exposed mice and rats to oxidative thermal degradation products of polystyrene and examined the effects on nonprotein sulfhydryl content and various xenobiotic-metabolizing enzymes both in liver and in lung.

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MATERIAL AND METHODS

Chemicals

Polystyrene (PS-RH) was obtained from Stymer Oy, Finland, and according to the manufacturer it contained no additives. 7-Ethoxycoumarin was synthesized according to Ullrich et al. (16). 7-³H-Styrene oxide (25–50 mCi/mmol) was obtained from Radiochemical Centre, England. All the other chemicals were of reagent grade.

Exposure

Mice. The exposure system used for mice is schematically presented in fig. 1. The exposure chamber was constructed from a 20-l desiccator. The inlet for plastic fumes was at the bottom and the outlet at the top of the chamber. Mice were placed in a stainless steel cage located 10 cm from the bottom. Granular calcium chloride was used in the chamber to keep the humidity within the proper range (60–80 %). Polystyrene was degraded in a J-shaped glass tube (inner diameter 7 mm), inserted through a hole into an oven heated to 350°C. The short arm of the tube was completely inside the oven to ensure the right degradation temperature. Polystyrene (150 mg) was placed in the tube, and air was sucked through it for 15 min at the rate of 0.8 l/min by a pump located behind the exposure chamber. The fumes were led to the chamber through glass tubes connected to each other by PTFE tubing. After the 15-min degradation the pumping was stopped for 20 min. Thereafter the chamber was flushed with

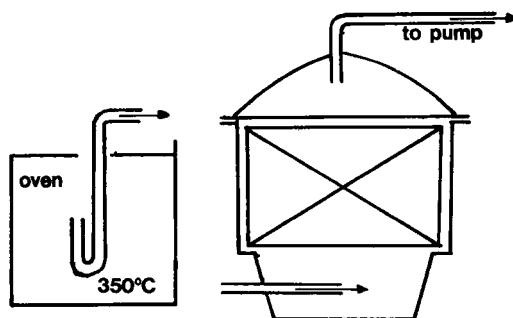


Fig. 1. Exposure system used for the mice.

fresh air for 10 min, and the procedure was repeated. Four doses were given in 1 d.

Rats. A schematic picture of the dynamic exposure system for rats is displayed in fig. 2. The plastic to be decomposed was placed in a glass tube (length 150 cm, outer diameter 17 mm). The plastic load was 0.1 g/cm. The tube passed through a tubular oven (diameter of the tube 18 mm, length 100 mm) which was moved along the tube by a step motor at the rate of 10 cm/h. The temperature of the oven was set so that the degradation temperature of the plastic was 350°C. The degradation products were conducted to the exposure chamber by air (0.5 l/min). The degradation tube was connected to the chamber with a T-tube (PTFE) to which also a stream of fresh air (5 l/min) was conducted to dilute and cool the fumes. An even distribution of the degradation products in the chamber was accomplished with a cylindrical plastic end piece having ten radial side holes and being connected to the PTFE tube. The exposure chamber

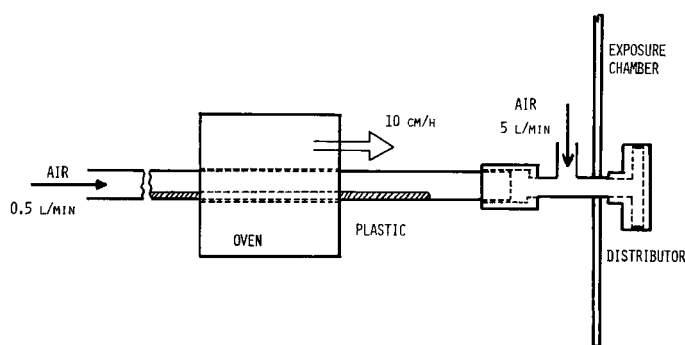


Fig. 2. Dynamic exposure system used for the rats.

(height 32 cm, width 50 cm, length 70 cm) was constructed from polymethylmetacrylate. The control animals were placed in a chamber with the same dimensions. The exposures were carried out at night, and a timer was used to start and stop the heating and movement of the oven. Rats were exposed for three weeks, 5 d a week, 6 h per night. The animals were decapitated 1 h after the last exposure.

Control of exposure levels. The exposure levels were controlled by a portable gas chromatograph (Model 511, Analytical Instrument Development, Inc., U.S.A.) equipped with an automatic sampler (Valveminder II, Carle, Inc., U.S.A.). The cycle time for sampling was 1 min. A gas loop was installed in the gas chromatograph. No column was used; so the FID detector responded to the total amount of combustible compounds in the chamber air. The gas chromatograph was calibrated with styrene. We generated suitable concentrations of styrene by injecting it into a laminated plastic bag and letting it equilibrate with 6 l of air. During the intermittent exposures for the mice the mean concentration was 150 ppm (expressed as styrene) (range 80–200 ppm). During the dynamic exposures the concentration was 140 ± 10 (SD) ppm after equilibrium was attained some 30 min after the beginning of decomposition.

Carbon monoxide concentrations were measured with Dräger tubes (Drägerwerk AG, Federal Republic of Germany), and they never exceeded 30 ppm. Humidity was below 80 %. A Beckman Oxygen Analyzer D2S (Beckman Instruments, Inc., U.S.A.) was used to measure the oxygen concentration, which was 19.5 ± 0.6 (SD) %. The temperature in the exposure chambers was 23–25°C.

Biochemical methods

NMRI mice (male, 20–25 g) and Wistar rats (male, 250–280 g) were kept on a standard diet. Food was not given during the exposures. The animals were killed by decapitation, their livers and lungs (only rats) were removed, and Ca^{++} -sedimented microsomes were prepared (2).

Microsomal protein was determined by the method of Lowry et al. (9) with bovine serum albumin as the standard.

Cytochrome P-450 of the microsomes was determined according to the method of Omura and Sato (13) with a Varian Cary 219 spectrophotometer. Microsomal O-dealkylation was measured with 7-ethoxycoumarin as the substrate by the method of Ullrich and Weber (16) as modified by Aitio (1). Diphenyloxazole (PPO) hydroxylation was assayed according to Cantrell et al. (3). The epoxide hydratase assays were carried out with the radiometric method of Oesch et al. (12) as modified by Vainio and Parkki (19). The activity of UDPglucuronosyltransferase was assayed at pH 7.4 with 4.5 mM UDPglucuronic acid and 0.35 mM p-nitrophenol as an aglycon (6, 17).

Glutathione S-transferase was determined with ^3H -styrene oxide substrate according to the procedure of Marniemi and Parkki (10).

The contents of the nonprotein sulfhydryl groups were selected to represent the concentrations of reduced glutathione (GSH) in tissues. The determinations were carried out by the fluorometric method of Hissin and Hilf (5) (mice) and by the colorimetric method of Saville (15) (rats).

RESULTS

Effects of thermal degradation products on mouse liver

The GSH levels were decreased to 70 % of those of the controls after a single 3-h exposure; 24 h after the cessation of exposure the GSH levels had been overcompensated by increased synthesis (table 1).

The cytochrome P-450 content in mouse liver decreased (15 %) during the first hours after acute exposure but reached the control level again in 2 d.

No significant change in the activity of an overall drug oxidation reaction, O-dealkylation of ethoxycoumarin, was observed during the first hours after the acute exposure. Within 24 h, however, the ethoxycoumarin O-deethylase activity had almost doubled (table 1).

Both hepatic cytochrome P-450 content and ethoxycoumarin O-deethylase activity were increased about twofold when the exposures were continued for 2 d. Cytochrome P-450 content remained at this level for 10 weeks, but the ethoxycoumarin O-deethylase enhancement gradually disappeared as exposure continued (table 2).

No exposure effect on microsomal epoxide hydratase activity could be detected in mouse liver.

Effects of intermittent subacute exposure to polystyrene fumes on rat liver and lung

Subacute intermittent exposure (three weeks) to polystyrene thermal degradation products depressed the hepatic GSH levels in rats only slightly. One hour after the last exposure the ratio of the hepatic GSH level in exposed rats versus the con-

trols was 95 % ($p < 0.001$), but soon this ratio increased (133 % 4 h and 121 % 24 h after the last exposure, $p < 0.001$). In rat lung the corresponding ratio 1 h after the last exposure was 80 % (table 3), the depletion of glutathione being greater than in liver.

Lung ethoxycoumarin O-deethylase activity was increased 1.5-fold after subacute exposure for three weeks. UDPglucuronosyltransferase activity was simultaneously depressed slightly (30 %) in the lungs.

In rat liver, cytochrome P-450 was increased almost twofold and ethoxycoumarin O-deethylase almost 1.5-fold by the subacute exposure (table 4). Another mixed-function oxidase, diphenyloxazole hydroxylase, was not affected. No significant changes in the activities of hepatic epoxide hydratase and glutathione S-transferase were detected. In UDPglucuronosyltransferase there was a slight (about 20 %) increase.

Table 1. Effects of single 3-h exposure to polystyrene decomposition fumes on reduced glutathione (GSH) levels, microsomal cytochrome P-450 content and ethoxycoumarin O-deethylase activity in mouse liver at different intervals after the cessation of exposure (means $\pm$ SD, 5 mice/group).

Hours after exposure	GSH (% of controls)	Cytochrome P-450 (nmol $\times$ mg protein ⁻¹)	Ethoxycoumarin O-deethylase (nmol $\times$ min ⁻¹ mg protein ⁻¹)
Controls	100	1.01 $\pm$ 0.07	1.57 $\pm$ 0.35
1	70	0.86 $\pm$ 0.05***	1.48 $\pm$ 0.14
4	84	0.87 $\pm$ 0.09**	1.57 $\pm$ 0.16
24	113	0.89 $\pm$ 0.10**	2.74 $\pm$ 0.31***
48	102	1.00 $\pm$ 0.13	2.51 $\pm$ 0.32***

** $p < 0.01$; *** $p < 0.001$.

Table 2. Effects of exposure to polystyrene thermal degradation products (3 h/d, 5 d/week) on xenobiotic-metabolizing enzymes in mouse liver (means $\pm$ SD, 5 mice/group).

Exposure	Cytochrome P-450 (nmol $\times$ mg protein ⁻¹)	Ethoxycoumarin O-deethylase (nmol $\times$ min ⁻¹ mg protein ⁻¹)	Epoxide hydratase (nmol $\times$ min ⁻¹ $\times$ mg protein ⁻¹)
Controls	0.58 $\pm$ 0.07	1.20 $\pm$ 0.24	17.9 $\pm$ 2.4
2 days	0.90 $\pm$ 0.04***	2.75 $\pm$ 0.18***	18.6 $\pm$ 2.0
4 days	0.94 $\pm$ 0.21***	2.00 $\pm$ 0.52**	—
2 weeks	1.29 $\pm$ 0.15***	1.92 $\pm$ 0.15***	17.2 $\pm$ 2.3
10 weeks	0.90 $\pm$ 0.16***	1.30 $\pm$ 0.14	18.8 $\pm$ 1.0

** $p < 0.01$; *** $p < 0.001$.

Table 3. Effects of subacute exposure (3 weeks, 6 h/night, 5 d/week) on xenobiotic-metabolizing enzymes and glutathione levels in rat lung (means $\pm$ SD, 4 rats/group).

Group	Glutathione ($\mu\text{mol/g}$ wet tissue)	Ethoxycoumarin O-deethylase ($\text{nmol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$)	UDPglucuronosyl- transferase ($\text{nmol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$)
Controls	1.86 ± 0.11	0.11 ± 0.01	0.95 ± 0.27
Exposed	$1.47 \pm 0.07^{***}$	$0.15 \pm 0.02^{**}$	$0.68 \pm 0.18^*$

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. Effects of polystyrene thermal degradation products on drug-metabolizing enzymes in rat liver after intermittent subacute exposure for three weeks (6 h/night, 5 nights/week) (means $\pm$ SD, 4 rats/group).

Enzyme	Controls	Exposed
Cytochrome P-450 ($\text{nmol} \times \text{mg protein}^{-1}$)	0.79 ± 0.11	$1.30 \pm 0.36^{**}$
Ethoxycoumarin O-deethylase ($\text{nmol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$)	1.13 ± 0.12	$1.52 \pm 0.12^{***}$
Diphenyloxazole hydroxylase (fluorescence units $\times \text{min}^{-1} \times \text{mg protein}^{-1}$)	127 ± 15	130 ± 30
Epoxide hydratase ($\text{nmol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$)	11.5 ± 1.0	13.8 ± 1.6
UDPglucuronosyltransferase ($\text{nmol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$)	1.87 ± 0.23	$2.27 \pm 0.31^*$
Glutathione S-transferase ($\text{nmol} \times \text{min}^{-1} \times \text{mg cytoplasmic protein}^{-1}$)	236 ± 9	221 ± 23

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

DISCUSSION

An acute 3-h exposure of mice to the complicated mixture of polystyrene decomposition products, containing, e.g., styrene, benzaldehyde, acetophenone, styrene oxide, 1-phenylethanol, and allylbenzene (14), caused a marked depression in the free nonprotein sulfhydryl groups of the liver. The depression was soon compensated by an increased synthesis of glutathione. The xenobiotic-induced glutathione depletion has been related to an increase in the toxicity of several compounds, and it may constitute a predisposition to toxicity (4). The changes we observed in glutathione levels after exposing animals to polystyrene thermal degradation products suggest that these products interact with endogenous nucleophiles. Styrene is known to cause a time- and dose-dependent decrease in hepatic GSH levels (18), and it is probably

also responsible for the effect observed in the present study. The increase in glutathione levels that occurred soon after the exposures ceased might represent a rebound phenomenon, observed after the administration of other chemicals as well (e.g., trichloroethylene, vinyl chloride) (11, 20).

The depletion of GSH in lung suggests an interaction of the decomposition products with cellular nucleophiles also in lung tissue.

The reason for the initial decrease in cytochrome P-450 content in mouse liver after an acute exposure is unknown, and it does not seem probable that the effect would be due to carbon monoxide because of its low concentration in the exposure chamber (below 30 ppm). When the exposure was continued, the cytochrome P-450 content increased.

Both acute and subacute treatments

were able to increase ethoxycoumarin O-deethylase activities in liver. After acute exposure the activity almost doubled within 24 h in mouse liver. In subacute experiments this enhancement gradually disappeared as the exposure continued. The reason for this effect is unknown, but it may be due to the accumulation of some thermal degradation product(s) or metabolite(s) that inhibit the mixed-function oxidase system.

Another overall monooxygenase reaction, hydroxylation of diphenyloxazole, was not affected by exposure for three weeks to the polystyrene decomposition fumes (table 4). The oxidation of diphenyloxazole has been proposed to be catalyzed by another type of P-450 hemoprotein, cytochrome P-448 (3).

It has been suggested that many foreign compounds are converted by microsomal monooxygenase to reactive electrophilic epoxides which can chemically modify important cell constituents and cause various adverse effects (7). The epoxides are inactivated by epoxide hydratase and conjugation with glutathione. In this study no significant effects on epoxide hydratase and the conjugating enzymes were detected (table 4). This lack of effect, accompanied by a simultaneous increase in the monooxygenase activity and depletion of reduced glutathione, may increase the possibility of interaction between the degradation products and cellular macromolecules.

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QUESTIONS AND ANSWERS

Question to Mr. Zitting

Dr. Rinzema:

You tested styrene polymer degradations at 350°C. This temperature is far above the practical polymer processing temperature. Most of the processing is done at $\approx 220^\circ\text{C}$. Injection molding may be done at a higher temperature, but seldom above 300°C. Don't you consider your tests as done at too high a temperature to simulate what workmen may possibly be exposed to? May not you find an entirely different kind of decomposition under conditions close to pyrolysis?

Mr. Zitting:

It is not easy to simulate the actual processing situation in the laboratory. For example, we have not, in our experiments, the elevated pressure which is used in the processing and very probably affects the decomposition mode. The temperature used (350°C) was a compromise. The number of different products is great, but the amount of inorganic products, especially carbon monoxide is low. We can also easily reach meaningful exposure levels without using large amounts of plastic. It is true that temperature has a great influence on the decomposition mode, and we must of course carry out the studies at other temperatures, too.