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MUTAGENICITY OF BUTADIENE AND BUTADIENE MONOXIDE

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SUMMARY : Incubation of S.typhimurium strains TA1530 and TA1535 in the presence of gaseous butadiene increased the number of his^+ revertants/plate. This mutagenic effect occured in absence of fortified S-9 rat liver fraction. In its presence, the mutagenic effect seemed to be dependent on its composition. With butadiene monoxide, a reversion to histidine prototrophy was obtained without metabolic activation with strains TA1530, TA1535 and TA100. Butadiene monoxide might be a possible primary metabolite of butadiene.

INTRODUCTION : 1,3-butadiene (vinyl ethylene, divinyl) is a widespread industrial compound. In particular, butadiene is widely used as a polymer component in the manufacture of synthetic rubber and copolymeric plastics such as ABS (Acrylonitrile-Butadiene-Styrene), regularly used for the packaging of foodstuffs.

Butadiene may consequently be considered as one of the prevalent environmental so called "monomers".

The recent demonstration of the carcinogenic [1,2,3,4] as well as the mutagenic[5,6,7] effects of vinyl chloride has drawn attention to the potential hazards associated with the use of those vinylic monomers.

Moreover, the mutagenicity of several other plastic monomers, such as vinylidene chloride[8], 2-chlorobutadiene [8], styrene [9,10,11] and acrylonitrile [12] is now clearly established.

The existence of a clear structural relationship between those mutagenic monomers and butadiene, which all contain vinylic groups prompted us to investigate the possible mutagenic effects of butadiene, to which humans are regularly exposed, especially by inhalation in industrial atmospheres.

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The interest of the present investigation was furthermore reinforced by the fact that two possible oxidative metabolites of butadiene, namely butadiene monoxide [13,14] and more especially diepoxybutane [13,14,15,16] possess carcinogenic properties ; moreover 1,2-epoxybutane and diepoxybutane have been proven to be mutagenic compounds in several test systems [17,18,19,20,21,22]. Finally, at least for some of those monomers, such as vinyl chloride[5] or styrene [10,11], their mutagenic properties are due to the formation of a reactive epoxide intermediate, under the influence of the microsomal mixed function oxidases.

MATERIALS AND METHODS

Chemicals : butadiene (purity 99.5 %) was obtained from Matheson Gas Products, Belgium. Butadiene monoxide (purity 97 %) was purchased from Aldrich Europe-Janssen Pharmaceutica, Belgium. Other products were of the purest grade available. Butadiene monoxide solution (1M) in ethanol (99.8 %) was stored in the dark at -18°C and under nitrogen. Dilutions were made in ethanol (99.8 %) before performing the mutagenesis assays.
Animals : adult male Wistar rats (200-250 g) were fed a RAL diet. Rats were injected i.p. (dosage : 500 mg/kg) with Arochlor 1254 diluted in cornoil (200 mg/ml) 5 days prior to the preparation of the liver fractions [23].
Mutagenicity assays : the S-9 fractions were obtained from 3 pooled rat livers, the homogenate (3 ml of 0.15 M KCL/g wet liver) of which was centrifugated as described [23]. Preparation of S-9 mix was made according to Ames and coworkers [23] by adding $MgCl_2$ (8 µmoles/ml mix), KCL (33 µmoles/ml mix), sodium phosphate (100 µmoles/ml mix), glucose-6-phosphate (5 µmoles/ml mix) and $NADP^+$ (4 µmoles/ml mix). 300 µl (75 mg wet liver/ml mix) or 200 µl (50 mg wet liver/ml mix) of the S-9 were utilized.
S.typhimurium strains TA1530, TA1535, TA1537, TA1538, TA98 and TA100 were kindly provided by Professor B.N.Ames.
Butadiene monoxide : tests were performed in duplicate by mixing substrate dilutions (0.1 mg/plate and 2-7 x 10^7 viable bacterial cells from an overnight culture in nutrient broth (Difco)/plate in histidine-biotin (0.05 mM) supplemented top agar (2 ml/plate) which was then layered on minimal glucose agar (Vogel Bonner E medium) in Petri dishes. After incubation of the plates for 48 hours at 37°C in the dark, the number of macroscopic colonies were counted.
Butadiene : tests were performed as follows : 2-7 x 10^7 viable bacterial cells from an overnight culture in nutrient broth (Difco)/plate and S-9 mix (0.5 ml/plate) were added to histidine-biotin supplemented top agar (2 ml/plate) which was then layered on minimal glucose agar in Petri dishes.

10 minutes later, the plates were opened and placed in a dessicator (30 l) equipped with inlet and outlet valves [24] and kept at 37°C in the dark.
Butadiene was continuously injected (total amount injected : about 20 l) through the inlet valve and the injection was stopped after one hour. The incubation in the presence of butadiene was then continued for various times.

The plates were withdrawn from the dessicator and incubated at 37°C in the dark until a total incubation time of 72 hours has elapsed.
The number of his^+ revertants was determined.
When carried out, the toxicity of butadiene was evaluated as follows : the bacterial survival was determined by operating as indicated above except that the bacterial inoculum was lower (10^5 fold dilution) and that top agar was poured on nutrient agar (Difco).
The concentration of butadiene in the dessicator atmosphere was evaluated by gas-liquid chromatography : a 20 µl sample of the gas effluent was taken off at the outlet valve of the dessicator and injected into a F 11 Perkin-Elmer gas chromatograph equiped with a flame ionization detector ; the column (0.4 x 200 cm) was packed with 10 % Apiezon L on 80-100 mesh chromosorb W and maintained at 40°C.

RESULTS AND DISCUSSION

Mutagenic effect of butadiene

A direct (in absence of S-9 mix) mutagenic effect was observed towards TA1530 and TA1535 strains ; after incubation for 20 hours in the presence of butadiene, the TA1537, TA1538, TA98, TA100 strains showed a number of his^+ revertants/plate corresponding to the spontaneous reversion rate (table 1).
In the presence of S-9 mix, a similar phenomenon was observed : a reversion to histidine prototrophy occured with TA1530 and TA1535 strains ; with the other ones, no mutagenic response was noticed (table 1).
However, the addition of a fortified S-9 fraction had contradictory effects upon the reversion rate : the number of his^+ revertants significantly decreased with S-9 amounts of 150 µl/plate and slightly increased with amounts of 100 µl/plate (table 1, figure 1).
Such a decreasing effect was unexpected and was for instance contrary to results obtained with vinyl chloride [25].
Investigations are in progress with a view to clearing up this effect which might reflect either the formation of a toxic metabolite or an unbalanced enzymatic action of the NADPH generating system (hydratase more active than oxidase i.e.) due to an inadequate composition of S-9 mix. The mix composition indeed seemed to influence the reversion rate and for instance was by no means optimal. Moreover, the fact that no significant toxic effect upon the bacterial cells was observed

TABLE 1. Mutagenic effect of butadiene on S.typhimurium strains in the presence and in the absence of a fortified S-9 fraction.

	No of his^+ rev./plate					
	Controls		Butadiene*		Butadiene*	
Fortified S-9 fraction	Omitted	added (300 µl S-9 /ml mix)[a]	Omitted	added (300 µl S-9 /ml mix)[a]	Omitted	added (200 µl S-9 /ml mix)[b]
Strains						
TA1530	13	13	80	43	58	65
TA1535	14	14	52	37	55	65
TA1537	7	20	11	10	15	13
TA1538	25	24	28	34	31	23
TA98	29	35	15	34	14	28
TA100	158	146	118	98	108	154

* Incubation time in the dessicator : 20 hours ; at that time, the percentage of butadiene in the dessicator atmosphere was about 70 %.

a incubation was performed as described in MATERIALS AND METHODS ; total time : 72 hours.

b incubation time was shorter ; after 20 hours incubation with butadiene in the dessicator, plates were incubated during a further 24 hours ; total time : 44 hours.

and that the mutagenicity curves obtained with and without S-9 mix had a similar aspect (figure 1) give support to this hypothesis.

Under our experimental conditions, the mutagenic effect of butadiene developed slowly : during the first hours, the reversion rate was very low and in spite of a large reduction in the percentage of butadiene in the atmosphere (about 25 %) the number of his^+ revertants was at the maximum after an exposure of about 24 hours (figure 1). This observation might be connected with the very weak solubility of butadiene in aqueous phase. Lastly, it must be pointed out that mutagenicy assays carried out with solutions of butadiene in ethanol by the usual method[23] of mixed substrate in top agar never showed an increased number of revertants as compared to the spontaneous mutation rate.

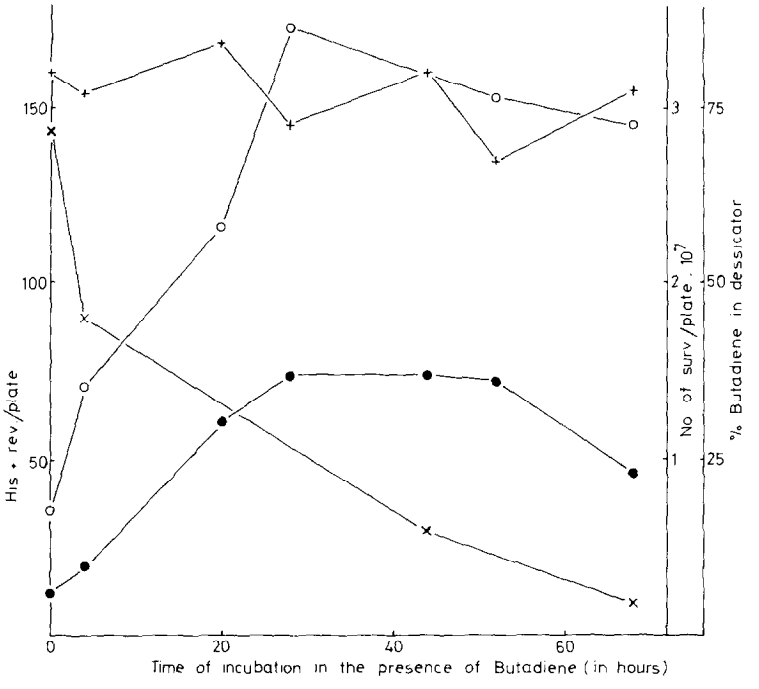

Fig.1. Mutagenicity assay of butadiene with strain TA1530. Total incubation time : 72 hours at 37° C. Bacterial inoculum : 0.1 ml of a 12 hours culture in nutrient broth (Difco)/ plate. Amount of S-9 in S-9 mix : 300 µl/ml mix.
O : S-9 mix- ; ● S-9 mix + ; + : No of surv./plate (x 10^7) ; X: Percentage of butadiene in dessicator.

Mutagenic effect of butadiene monoxide

For concentrations of butadiene monoxide ranging from 1 µmole up to 250 µmoles/plate, there was no significant effect upon TA1537, TA1538 and TA98 strains. Reversion to histidine prototrophy occured only with TA1530, TA1535 and TA100 strains (figure 2). The mutagenic effect was observed with TA100 within a range of 1 µmole /plate to beyond 250 µmoles/plate and with TA1535 and TA1530 within a range of 1 µmole/plate to 100 µmoles/ plate. The effect was especially pronounced within a range of 10 µmoles/plate to 100 µmoles/plate.
The maximal number of his^+ revertants/plate was observed for concentrations of about 100 µmoles/plate. TA1530 and TA1535 seemed to be the most sensitive strains : the mutation rate was tenfold the spontaneous reversion rate whereas with TA100,

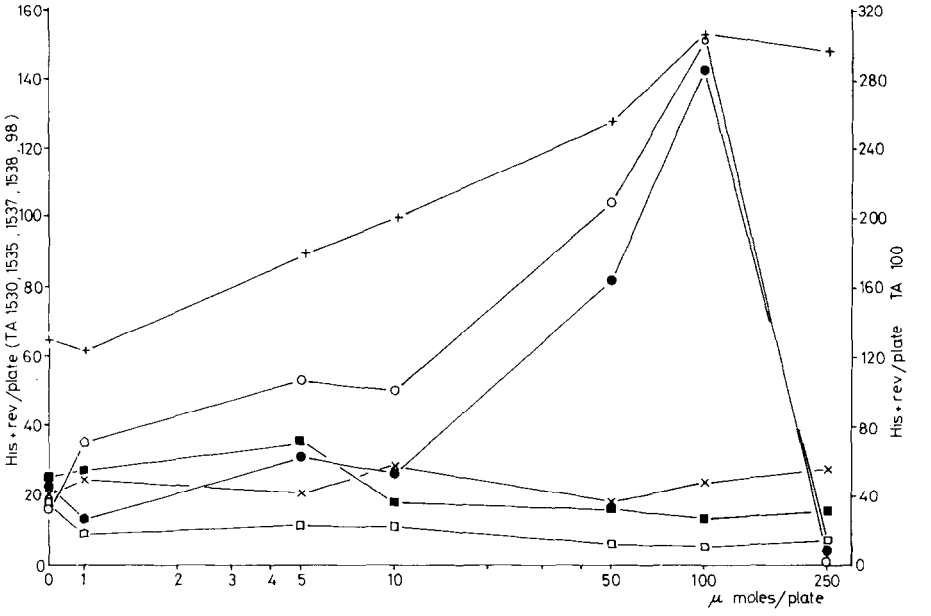

Fig.2. Mutagenicity assay of butadiene monoxide with S.typhimurium strains in the absence of S-9 mix.
O : TA1530 ; ● : TA1535 ; □ : TA1537 ; ■ : TA1538 ; ✖ : TA98 ; + : TA100.

it was only twofold the spontaneous reversion rate.
The number of TA1530 and TA1535 revertants abruptly decreased at butadiene monoxide concentrations of 250 μmoles/plate. It must be noted that such a toxic effect was observed only with the two sensitive strains. Similarities exist between the mutagenicity curves of butadiene monoxide and styrene oxide[11] ; moreover the sensitivity of TA100 towards both oxides is noticeable. The selective mutagenic effect of butadiene and butadiene monoxide in certain S.typhimurium strains, such as TA1530, TA1535 and TA100 (this latter with butadiene monoxide only), indicates that base-pair substitutions in the bacterial DNA are involved.
Ouring to the concentrations of the chemicals used in the experiments and the number of revertants detected, the mutagenic response may be considered as relatively weak.
Since the same strains (TA1530, TA1535) are sensitive to both butadiene and butadiene monoxide and because a similarity exists between the mutagenic properties of butadiene and vinyl chloride[25]

(existence of a mutagenic effect in absence of a metabolic activating system, influence of the mix composition upon the reversion rate), it is not irrealistic, by analogy, to speculate that butadiene monoxide is a probable primary metabolite of butadiene.

Other possible mechanisms of activation, such as radical formation or oxidative metabolism by the bacteria themselves are not yet excluded. Experiments are in progress to try to elucidate that point.

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