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ORIGINAL PAPERS

METABOLIC INTERACTION AND NEUROLOGICAL EFFECT OF COMBINED EXPOSURE TO ACRYLAMIDE AND ETHANOL

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Abstract. The assumption that interaction of ethanol and acrylamide with microsomal monooxygenase system may modify the neurotoxic effect of acrylamide was verified experimentally in rats. Animals pretreated for 5 weeks with ethanol (10% water solution as sole drink) were given acrylamide (30 mg/kg/ /day, 5 days a week) for the next 3 weeks in combination with ethanol. It was revealed that acrylamide induced functional disorders and morphological damages in the peripheral and central nervous system. The abnormally slow motor conduction velocity, the loss of ability to perform on rotarod, axons degeneration of a "dying back" nature in peripheral nerves in rats given a total cumulative dose of 450 mg/kg of acrylamide were found. In the combined exposure to ethanol and arcylamide the stimulating effect of ethanol on microsomal monooxygenase system was attenuated, whereas the noxious effect of acrylamide on the peripheral and central nervous system was aggravated.

INTRODUCTION

Since acrylamide is metabolized and detoxified in the hepatic microsomal enzyme system (1,5), it was assumed that the neurotoxicity of acrylamide may be related to the fate of its biotransformation in this system and some factors affecting the cyt. P-450 monooxygenases may modify its neurotoxic effect. The finding that rats pretreated with phenobarbital required a higher dose of acrylamide to produce functional impairments to the central nervous system supported this hypothesis (9). On the basis of the above concept, the supposition has been made that ethanol may influence arcylamide toxicity. Ethanol stimulates microsomal cyt. P-450 monooxygenases (17,18) and our recent studies have

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indicated that ethanol potentiated CS_2 chemical's neurotoxicity (11,12) which undergoes biotransformation in microsomal cyt. P-460 system (17,18). In the current study, the interaction of ethanol and acrylamide in the microsomal system and the effect of ethanol intake on produced by arcylamide neurotoxicity have been studied.

MATERIAL AND METHOD

Rats pretreated for 5 weeks with ethanol (10% water solution as sole drink) for the next three weeks jointly with ethanol, were given 15 doses of acrylamide (30 mg/kg/day, 5 days a week by gaster gavage). Reference groups of rats drank water or ethanol only and were kept in the same condition. At the end of the eighth week of experiment (24 hours after last administration of acrylamide), estimation of cyt. P-450 monooxygenases and glutathione S-transferase (GHS-S-transferase) in liver, as well as functional and morphological evaluation of central and peripheral nervous system were carried out.

Biochemical assays: methods used in the preparation of microsomes, determination of microsomal cyt. P-450 monooxygenases, GSH-S-transferase and non-protein sulfhydryl, content in the liver were described in our previous paper (18). Functional tests: evaluation of functions of the central nervous system (CNS) and peripheral nervous system (PNS) was done with rotarod performance test and motor conduction velocity measurement. Rotarod performance was measured similarly to the method described by Kaplan and Murphy (8). Motor conduction velocity in sciatic and tibial nerves was recorded on Hewlett-Packard electromyograph acc. to Knobloch et al. (10). Morphological examination: ultrastructure of hepatocytes after standard procedure was examined under JOEL JEM-100C electron microscope; the light microscopy of CNS was performed in paraffin sections and peripheral nerves in "semithin" — epon sections.

One-directional variance analysis was applied for statistical evaluation of the results.

RESULTS

Evaluation of liver toxicity

A cumulative dose of acrylamide (450 mg) did not induce any changes in cyt. P-450 monooxygenases. However, under the influence of acrylamide the stimulatory effect of ethanol on cyt. P-450 enzymes was suppressed. Neither ethanol nor acrylamide, applied separately or in combination, affected the activity of the GSH-S-transferase (Table 1). The level of non-protein sulfhydryls in the liver was not altered (Table 1).

TABLE 1. Cytochrome P-450 Monooxygenase and GSH-S-transferase and Non-protein Sulfhydryls in the Liver of Rats Exposed ot Ethanol and Acrylamide (Singly and Combined)

Groups	Cyt. P-450 nmol/mg microsomal protein	Aniline p-hydroxylase nmol p-aminophenol 15 min/mg microsomal protein	GSH-S-transferase μmol/min/g liver	Non-protein sulfhydryls μmol/g liver
Control	0.56±0.06	13.6±1.1	50.2±1.6	6.6±0.3
EtOH	0.78±0.05[a]	21.5±1.5[b]	48.8±8.0	5.9±0.6
Acrylamide	0.54±0.06	15.9±0.8	49.5±6.9	7.2±0.3
EtOH+acrylamide	0.56±0.06	17.2±1.5	57.8±4.5	6.5±0.3
Phenobarbital reference control	1.56+0.09[c]	23.6+1,9[c]	109,8+2.2[c]	6.0±0.3

Regimen of exposure: 10% ethanol in drinking water for 8 weeks; Acrylamide — 0.3% (w/v) solution in water by gavage 5 times weekly at doses of 30 mg/kg body wt. for 3 weeks starting from 5th week of ethanol exposure; Phenobarbital — 0.1% solution in drinking water for 7 days.

Results are the mean + S.E. for 8—10 rats.

a, b, c Significantly different from the control at P=0.05, 0.01 and 0.001, respectively.

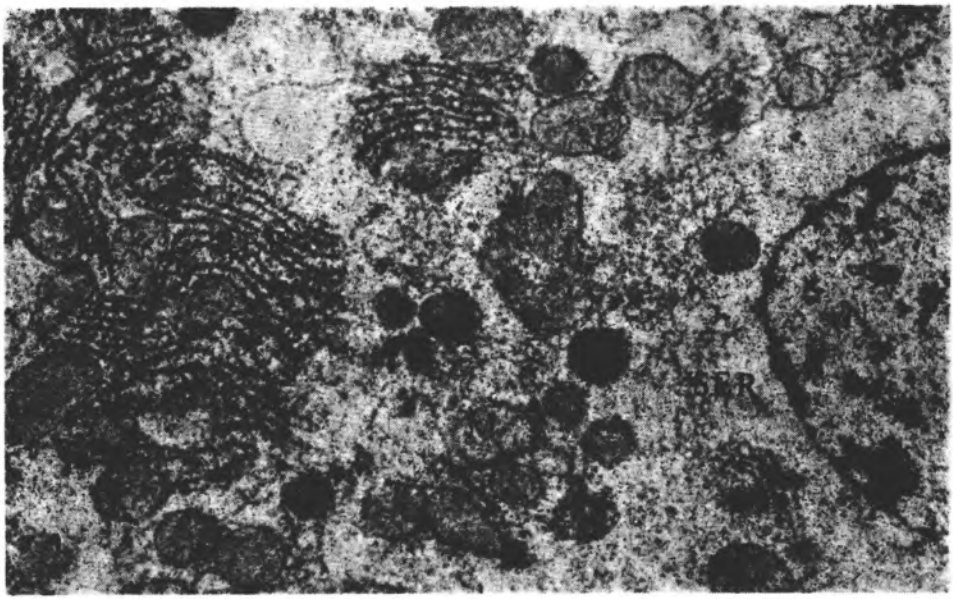

Fig. 1. Hepatocyte of control rat. Mitochondria (M), fragment of nucleus (N), smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER). Magn. 9.900 ×.

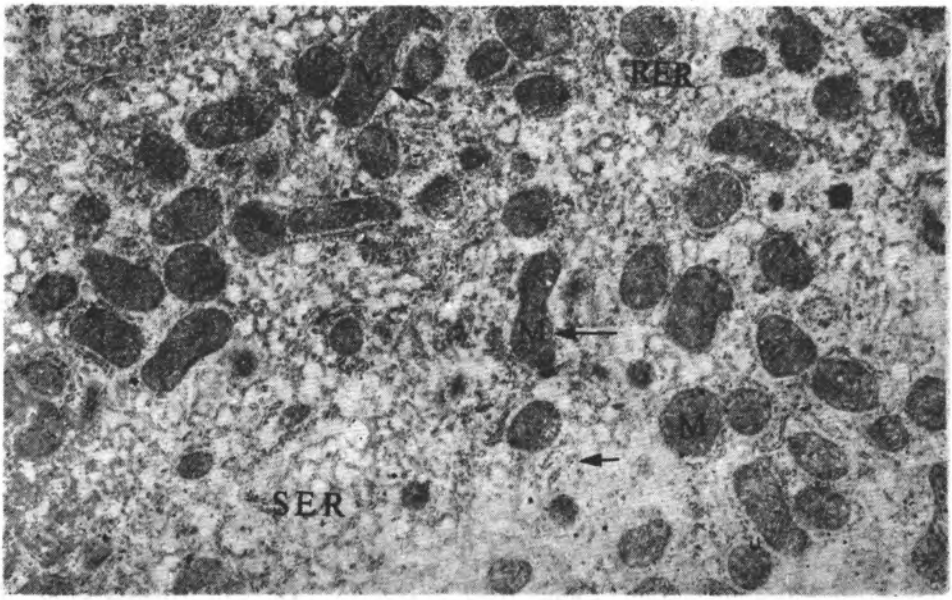

Fig. 2. Hepatocyte of rat exposed to 10% ethanol in drinking water for 8 weeks. Distortion in shape of some mitochondria (arrow). Slight proliferation of SER and signs of partial degranulation of RER (arrows). Magn. 9.900 ×.

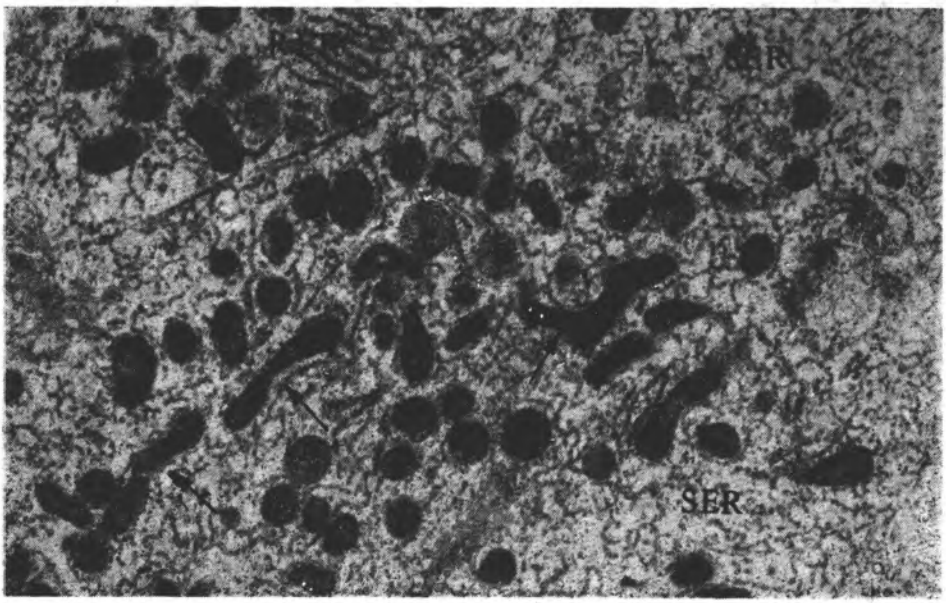

Fig. 3. Hepatocyte of rat exposed to acrylamide for 3 weeks (30 mg/kg). Small condensed mitochondria, some of them in the phase of dividing (arrows), numerous peroxisomes (P). Proliferation of SER and short segments of RER distributed in the cytoplasm. Magn. 9.900 ×.

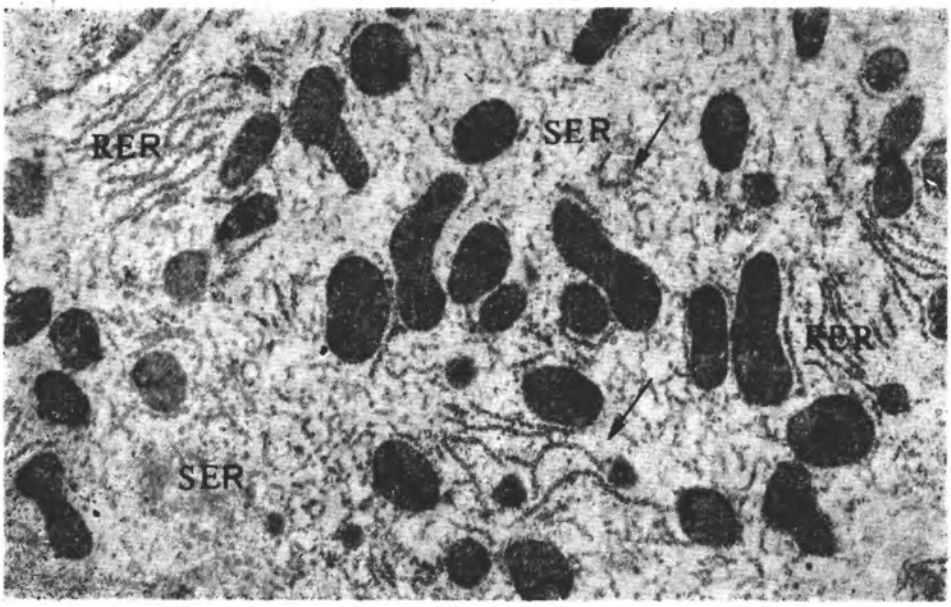

Fig. 4. Hepatocyte of rat jointly exposed to ethanol (8 weeks) and acrylamide (3 weeks). Distortion in shape of some mitochondria and normalised mitochondria. Proliferation of SER, several short fragments of RER (arrows). Magn. 9.900 ×.

Morphological examination showed that acrylamide as well as ethanol caused some ultrastructural alteration of hepatocytes. Slight prolliferation of smooth endoplasmic reticulum (SER) and signs of partial degranulation of rough endoplasmic reticulum (RER) were seen both in hepatocytes of rats treated with ethanol and acrylamide separately (Fig. 1—3). In rats treated with ethanol and acrylamide jointly, the amount of SER and RER determined qualitatively was similar to those observed in acrylamide intoxicated rats (Fig. 4). Distortion in shape of some mitochondria was seen in the hepatocytes of rats exposed to ethanol singly or in combination with acrylamide (Fig. 2—4).

Evaulation of neurotoxicity

Peripheral nervous system — after 15 doses of acrylamide given per os at rate 30 mg/kg/day, the rats showed distinct peripheral neuropathy: weakness of hind limbs and slowing motor conduction velocity. Acrylamide-induced impairment of motor conduction velocity was more pronounced in rats jointly treated with ethanol and acrylamide (Table 2). Morphological examination indicated the signs of axonal degeneration (disrupted axons and myelin ovoids) in the sciatic and tibial nerves of rats injected with acrylamide and acrylamide and ethanol jointly. The charac-

TABLE 2. Conduction Velocity in Peripheral Nerves of Rats Exposed to Ethanol and Acrylamide (Singly and Combined)

Groups	Motor conduction velicity in sciatic and tibial nerves m/sec
Control	51.0±1.9
EtOH	52.0±1.4
Acrylamide	41.2+1.5[c]
EtOH + acrylamide	36.7+0.98[cA]

Details are given in Table 1.
Results are the mean ± S.E. for 12 rats.
[c] Significantly different from control and EtOH groups at P = 0.001.
[A] Significantly different from acrylamide group at P = 0.05.

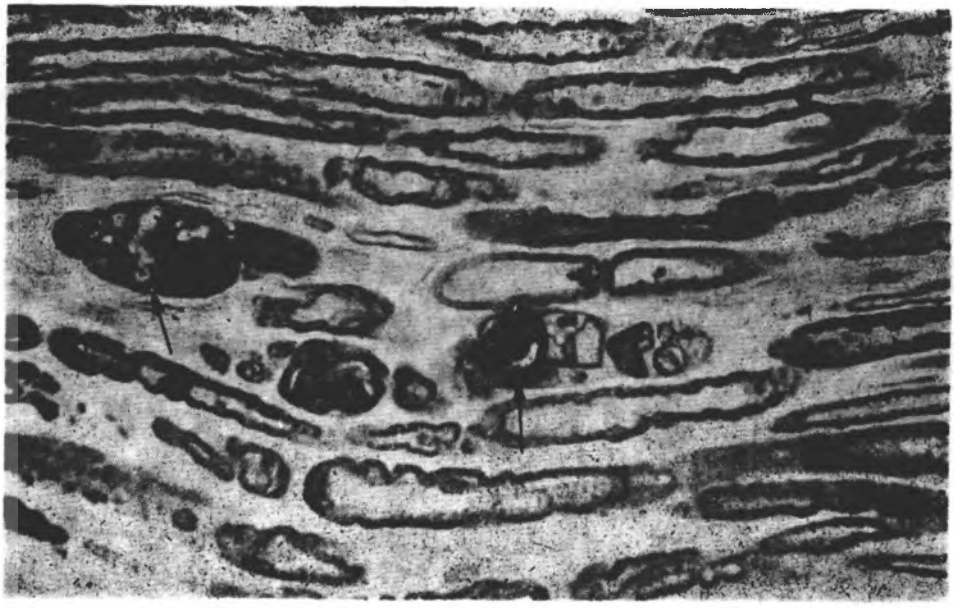

Fig. 5. Tibial nerve of rat exposed to acrylamide (3 weeks). Axonal degeneration — disrupted myelin fibres and myelin ovoids (arrows). The severity and character of changes in rats jointly exposed to ethanol and acrylamide were the same. Epon "semithin" sections, toluidine blue staining. Magn. 900 ×.

ter of peripheral axonopathy induced by acrylamide as well as by acrylamide and ethanol jointly was qualitatively the same; quantitatively, the degree and the extent of alterations could not be distinguished (Fig. 5).

Central nervous system — the rats treated with acrylamide exhibited ability of performance on rotarod lower than control animals. Ethanol administration aggravated disturbances produced by acrylamide in

TABLE 3. Rotarod Performance Ability as an Index of Neurotoxic Effect of Exposure to Acrylamide and Ethanol (Singly and Combined)

Gropus	Time of maintaining balance on rotarod (sec)			
	Number of doses of acrylamide			
	0	5	10	15
Control	52.7±13.2	52.8±13.3	58.2±14.3	62.5±14.8
EtOH	52.1±13.1	55.7±16.0	60.2±15.9	58.6±16.5
Acrylamide	49.2±12.7	70.0±14.9	49.6±15.4	28.4±11.5
EtOH + acrylamide	51.3±12.5	67.3±14.1	37.3±14.1	21.1± 5.6[a]

Details are given in Table 1.
Results are the mean ± S.E. for 12 rats.
[a] Significantly different from control and EtOH groups at $p = 0.05$

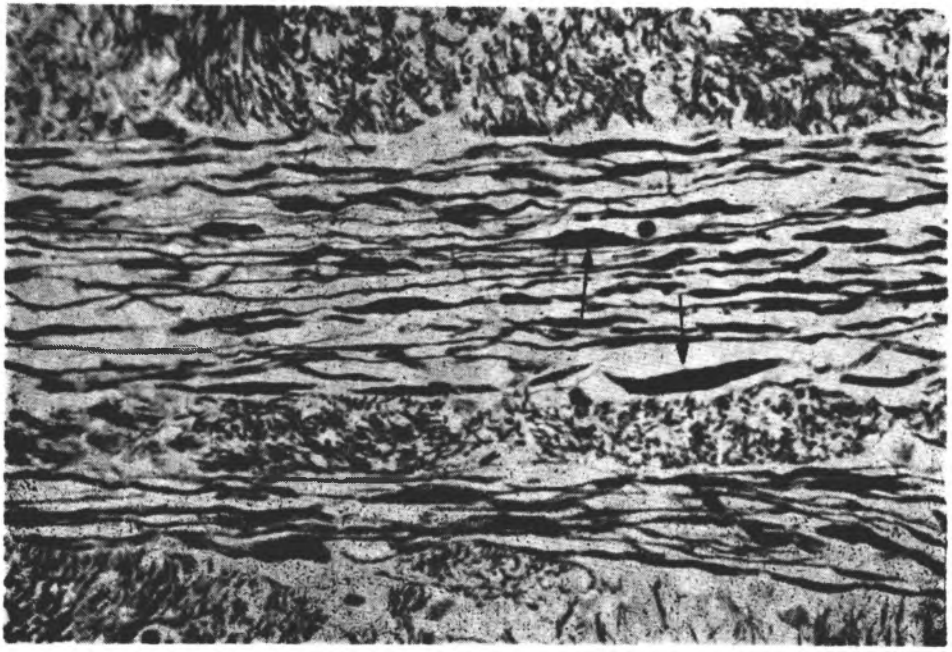

Fig. 6. Medulla oblongata of rat jointly exposed to ethanol (8 weeks) and acrylamide (3 weeks). Swollen axons in the medial lemniscus (arrows). In nervous tract of rats given singly ethanol or acrylamide no lesions were seen. Parafin sections; Gless-Marsland staining. Magn. 600 ×.

performance ability. In rats pretreated with ethanol, a shorter time and lower cumulative dose of acrylamide (300 mg/kg and 10 days) was needed for the onset of functional disorders (Table 3). Morphological examination of CNS indicated in the range of medial lemniscus and decussatio lemniscorum in pons a number of swollen agrophilic axons (Fig. 6).

DISCUSSION

Functional and produced by acrylamide morphological impairments of the central and peripheral nervous system and found in this study were consistent with those observed in various animals by many authors (2,3,4,7,8,9,13,15). In our experiment, a cumulative dose of acrylamide to produce sings of neuropathy was in the range 300—450 mg/kg. After 15 doses of acrylamide given per os at rate 30 mg/kg/day rats showed distinct peripheral neuropathy: weakness of hindlimbs and significant slowing of motor conduction velocity in the sciatic and tibial nerves at 25% in comparison with control.

A microscopic examination of the sciatic and tibial nerves showed sings of axonal degeneration — a number of fragmented fibres with ovoid formation but the majority of axons remained normal. Ultrastructural examinations of hepatocytes revealed that acrylamide had an effect on cytoplasmic membranes. The results, not reinforced by morphometric analysis, however, indicated a decrease of RER and an increase of SER. These data could be admitted as an indirect argument that biotransformation of acrylamide and combined acrylamide and ethanol activated some cellular structures (SER) which are the basis for many microsomal enzymes (16). On the other hand, the disorders seen in mitochondria (shrinkkage and increased density) and the increased number of peroxisomes may indicate the non-specific reaction pattern in the alarm phase of cell adaptation during acrylamide intoxication alone (14). Similar disorders were also noted in phenobarbital and cycloheximide intoxication (14).

As shown by Kaplan et al. (9), neurotoxicity of acrylamide may be influenced by many factors, among others by factors influencing microsomal enzymes: phenobarbital, DDT, SKF. Phenobarbital leads to a marked delay in the onset of functional neurological disorders in rats given acrylamide. According to Kaplan et al. (9) the mechanism of this effect results from an increased phenobarbital ability of the liver microsomal system to detoxify acrylamide. Since acrylamide is detoxified through its biotransformation in microsomal monooxygenases, it is possible that phenobarbital by stimulation of microsomal enzymes may reduce the neurotoxic effect of acrylamide.

Our experiments revealed that apart from phenobarbital, the neurotoxicity of acrylamide may be modified by ethanol. In contrast to phenobarbital (6,9) ethanol given to rats intoxicated with acrylamide pronounced its neurotoxicity as inferred from functional tests (loss of ability of rats to maintain balance on rotating rod and slowing of motor conduction velocity) and morphological examination of the nervous system (swollen axons in CNS were visible only in joined exposure).

The difference in modification of neurotoxicity of acrylamide produced by this chemical in combination with phenobarbital of ethanol may be due to the specificity and extent of phenobarbital of ethanol induction of the enzyme systems involved in the biotransformation of acrylamide. Biotransformation of acrylamide comprises two phases: first — oxygenation to epoxides via cyt. P-450 monooxygenases (bioactivation) and second — conjugation with non-protein sulfhydryls via GHS-S--transferases (detoxification).

Phenobarbital induces both cyt. P-450 monooxygenases and GSH-S--transferase, whereas the stimulatory effect of ethanol is limited to cyt. P-450 monooxygenases only (cf. Table 1 and 2). It might be assumed that delay in the onset of neurotoxic symptoms of acrylamide in phenobarbital-preinduced rats results from stimulation of both steps of its metabolism, especially from a two-fold increase in the activity of GSH--S-transferase, catalysing the detoxication step via conjugation of electrophilic metabolites with glutathione (cf. Table 2). Ethanol does not stimulate the activity of the GHS-S-transferase. Thus, the activation step in the acrylamide metabolism stimulated by ethanol is not compensated by the detoxification step mediated via GHS-S-transfer ase.

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