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# Effects of Short-Term Inhalation Exposure to 1-Nitropropane and 2-Nitropropane on Rat Liver Enzymes

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Male Sprague–Dawley rats were exposed to vapors of 1-nitropropane (1-NP) and 2-nitropropane (2-NP) at air concentrations of 100 ppm for 7 hours per day on four consecutive days. Livers were analyzed for enzymatic activities after 1-, 2-, and 4-day inhalation periods. Liver microsomal cytochrome P450 was depressed by 2-NP and elevated following exposure to 1-NP. Levels of cytochrome $b_5$ were slightly increased in rats exposed to 1-NP and remained unchanged after inhalation of 2-NP. Total glutathione (GSH), GSH *S*-transferase, and UDP-glucuronosyltransferase activities were enhanced by 2-NP. 1-NP induced GSH peroxidase while 2-NP did not. Glutathione reductase was not altered after exposure to either isomer. No changes in the microsomal malondialdehyde content as a measure of lipid peroxidation and in the levels of serum aspartate transferase and serum glutamic oxaloacetic transaminase were observed during a 4-day exposure period in either of the exposed groups compared to control animals. 

## INTRODUCTION

1-Nitropropane (1-NP) and 2-nitropropane (2-NP) are industrial solvents used mainly for coatings, for inks, and in separation processes (Baker and Bollmeir, 1978). Thus, the most likely occupational exposure is by inhalation. Both compounds are hepatotoxic regardless of the route of administration (Dequidt *et al.*, 1972). Several studies on the toxic effects of 2-NP (Griffin *et al.*, 1978, 1982; Lewis *et al.*, 1979) and 1-NP (Griffin *et al.*, 1982) on rats have been reported. Chronic exposure of rats to 2-NP at air concentrations of 100 ppm or higher resulted in severe hepatotoxicity and could lead to the formation of hepatocarcinomas (Griffin *et al.*, 1978). 1-NP did not cause any pathological effects in a near lifetime exposure of rats at 100 ppm (Griffin *et al.*, 1982). In agreement with this different toxicological behavior, 2-NP has been demonstrated to be genotoxic in rat hepatocytes, while 1-NP was ineffective (Andrae *et al.*, 1988).

To date, it is still unclear which enzymatic activities are involved in the metabolism and possibly the bioactivation of nitroparaffins. The cytochrome P450 monooxygenase system has been implicated in the conversion of 2-NP to acetone and nitrite by liver microsomes from rats and mice (Ullrich *et al.*, 1978; Marker and Kulkarni, 1985). The same metabolites were observed after administration of 2-NP to rats and chimpanzees (Müller *et al.*, 1983) as well as in incubations of rat hepatocytes and V79 cells with 2-NP. In addition, both cell lines were found capable of reducing 2-NP to acetone oxime (Haas-Jobelius *et al.*, 1991). In rats and chimpanzees 1-NP is metabolized to polar propionic acid derivatives (Haas-Jobelius *et al.*, 1989). *In vitro*, 2-NP and to a lesser degree 1-NP were found to be conjugated to glutathione (GSH) by GHS *S*-



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transferases purified from rat liver (Habig *et al.*, 1974). Zitting *et al.* (1981) observed a rapid increase of the hepatic GSH content as well as effects on several phase I and phase II enzyme activities of rat liver after intraperitoneal administration of 50 mg/kg 2-NP. These authors described the overall effects as indicative of lipid peroxidation in the liver.

The present study was conducted to establish whether 1-NP exerts similar effects on the activities of rat liver enzymes compared to its isomer 2-NP. Inhalation of both compounds at air concentrations of 100 ppm was chosen as the route of administration to allow for a simulation of conditions possibly met at the workplace.

## MATERIALS AND METHODS

### *Animals*

Male Sprague–Dawley rats (240–280 g body weight) were obtained from Sasco Inc. (Omaha, NE). The animals were divided into a control and two exposure groups and they were housed individually in galvanized iron cages.

### *Test Substances*

1-NP and 2-NP were obtained from International Minerals and Chemical Corporation or from Angus Chemical Company.

### *Exposure Conditions*

The test groups were exposed to vapors of 1-NP and 2-NP at 100 ppm for 7 hours per day on four consecutive days in exposure chambers (8 ft wide, 8 ft high, and 12 ft long) with controlled temperature and humidity. The control animals were housed in a room with environmental conditions similar to those in the exposure chambers. The concentrations of 1-NP and 2-NP vapors in the exposure chambers were monitored at least four times a day using an ir gas analyzer Model Miran IA (Wilks Scientific Corporation). The details of the exposure conditions and chamber operations have been previously reported (Griffin *et al.*, 1982).

### *Serum Analysis and Liver Preparation*

Ten minutes after termination of the exposure periods, the animals were removed from the chambers. On inhalation Days 1, 2, and 4, three animals from each of the exposed groups and three control animals were immediately sacrificed by decapitation. Livers were obtained and prepared as described below. After the fourth day exposure period serum samples were analyzed using commercial test kits (Statzyme GOT and Statzyme GPT from Worthington). Liver samples for enzymatic analysis were homogenized (25% w/v) in ice-cold 0.01 *M* phosphate buffer (1.15% KCl, pH 7.4) with a Potter–Elvehjem mortar and a Teflon pestle. After centrifugation at 10,000*g* for 30 min the supernatant was centrifuged at 105,000*g* for 60 min. The resulting soluble fraction was used for the determination of GSH-dependent enzyme activities. The microsomal pellet was resuspended in 5 ml of 1.15% KCl, recentrifuged at 105,000*g*, and suspended in 0.05 *M* phosphate buffer (1 mM EDTA, pH 7.4). For the determination of GSH, 2 ml of liver homogenate was deproteinized by adding 1 ml of 10%

trichloroacetic acid. The clear supernatant obtained after centrifugation was used for GSH analysis.

*Biochemical Analysis*

Microsomes were analyzed for cytochrome P450 and cytochrome $b_5$ using the method of Omura and Sato (1964) as modified by Mazel (1981). UDP-glucuronosyltransferase was determined with 1-[$^{14}$C]naphthol as a substrate (Halinan, 1984). Malondialdehyde was measured using the assay of Ohkawa *et al.* (1979). The supernatant fraction was used for the determination of GSH *S*-transferase with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Habig *et al.*, 1974) using the glutathione (GSSG) reductase method. The method of Reddy *et al.* (1981) was modified for analysis of GSH peroxidase in the supernatant. The assay mixture consisted of 2.5 m*M* GSH, 2.8 m*M* cumene hydroperoxide, 0.3 units of GSSG reductase (Boehringer-Mannheim), and 2.4 m*M* NADPH in 0.1 *M* Tris/HCl (2.8 m*M* EDTA, pH 7.0). Total GSH was measured by the Tietze method (1969) and protein according to Lowry *et al.* (1951). Statistical evaluations were performed using Student's *t* test.

## RESULTS

1-NP raised significantly hepatic cytochrome P450 by 22 or 28% after the 1- or 2-day exposure period compared to the controls. On the fourth day, however, no significant difference between control and exposed animals could be observed. The levels of cytochrome $b_5$ exhibited essentially the same time profile after exposure to 1-NP. In contrast, 2-NP caused a significant reduction of cytochrome P450 throughout the exposure phase by 45 on Day 1 and 39% on Days 2 and 4, while no influence on cytochrome $b_5$ could be detected (Table 1, a and b).

The activity of microsomal UDP-glucuronosyltransferase remained unchanged following inhalation of 1-NP and after the first 2 days of exposure to 2-NP. After the fourth day, levels were increased by 62% (Table 1, c). Values of microsomal malondialdehyde as a measure for hepatic lipid peroxidation were not altered following exposure to 1-NP and 2-NP (Table 1, d).

Whereas 1-NP had no effect, 2-NP treatment led to an induction of the hepatic GSH content. Due to the large interindividual variations in the values of Days 1 and 2, this effect was significant only on Day 4 with an increase of 47% (Table 2, a).

Both isomers enhanced the cytosolic GSH *S*-transferase activities. In the case of 1-NP, this effect was not significant while elevation was 39, 72, and 47% on Days 1, 2, and 4 for 2-NP (Table 2, b). Activities of cytosolic GSH peroxidase were increased by 33 and 41% after 1 and 2 days of exposure to 1-NP. After the fourth inhalation period, however, the values did not differ significantly from controls. An influence of 2-NP on GSH peroxidase could not be determined (Table 2, c). No differences in the levels of cytosolic GSSG reductase were observed between exposed and unexposed rats (Table 2, d).

Likewise, levels of serum glutamic oxaloacetic transaminase (GOT) and aspartate transferase after 4 days of exposure did not differ significantly from controls. (GOT: control 167 ± 56, 1-NP 147 ± 21, and 2-NP 185 ± 31 U/liter; GPT: control 30 ± 3, 1-NP 30 ± 2, and 2-NP 31 ± 2 U/liter).

TABLE 1

EFFECTS OF INHALATION OF 1-NP AND 2-NP ON RAT LIVER MICROSOMAL CYTOCHROME P450, CYTOCHROME $b_5$, UDP-GLUCURONOSYLTRANSFERASE, AND MALONDIALDEHYDE

| Day | Control | 1-NP | 2-NP |
|---|---|---|---|
| | a. Cytochrome P450 (pmol cytochrome P450/mg protein) | | |
| 1 | 550 ± 43 | 671 ± 39* | 300 ± 35* |
| 2 | 577 ± 14 | 737 ± 15* | 351 ± 125* |
| 4 | 526 ± 73 | 563 ± 209 | 320 ± 64* |
| | b. Cytochrome $b_5$ (pmol cytochrome b5/mg protein) | | |
| 1 | 385 ± 54 | 536 ± 28* | 452 ± 39 |
| 2 | 360 ± 6 | 447 ± 28* | 396 ± 105 |
| 4 | 433 ± 32 | 472 ± 85 | 420 ± 72 |
| | c. UDP-glucuronosyltransferase (nmol 1-napththol conjugated/min/mg protein) | | |
| 1 | 41.9 ± 12.2 | 35.9 ± 5.0 | 41.2 ± 0.9 |
| 2 | 50.8 ± 5.3 | 41.5 ± 6.2 | 44.4 ± 1.2 |
| 4 | 41.4 ± 1.9 | 41.6 ± 2.9 | 67.2 ± 4.6* |
| | d. Malondialdehyde (pmol/mg protein) | | |
| 1 | 791 ± 68 | 932 ± 150 | 854 ± 93 |
| 2 | 824 ± 33 | 862 ± 35 | 774 ± 79 |
| 4 | 856 ± 104 | 875 ± 161 | 777 ± 105 |

* Significantly different from controls at $P < 0.05$.

## DISCUSSION

The about 40% decrease of the cytochrome P450 content in rats exposed to 2-NP compared to the controls may reflect an inactivation of the monoxygenase system by 2-NP or a metabolic product and has also been observed by Zitting *et al.* (1981). Studies by Ivanetich *et al.* (1978) indicated that 2-NP degrades the heme moiety of microsomal cytochrome P450 upon metabolic activation. Similar observations have been reported by Porter and Bright (1983), who found that the nitronate form of 2-NP irreversibly inactivates the heme enzyme horseradish peroxidase presumably by covalent binding to the porphyrin ring system.

In contrast to the decrease of cytochrome P450 after exposure to 2-NP, 1-NP leads to a slight (22–28%) induction. Possibly, an intermediate which is capable of inhibiting cytochrome P450 is not formed in the case of 1-NP. This may indicate fundamental differences in the metabolism of both nitropropane isomers.

In agreement with the results of Zitting *et al.* (1981), an increase of UDP-glucuronosyltransferase activities by 62% was observed following inhalation of 2-NP, however, not after exposure to 1-NP. Compared to GSH *S*-transferase, GSH peroxidase, and cytochrome P450, the induction of UDP-glucuronosyltransferase activity follows a relatively slow time course with a significant elevation only after the fourth inhalation period. Exposure to 1-NP had no effect on the GSH content of rat liver, while an increase up to 47% was determined following exposure to 2-NP.

TABLE 2

EFFECTS OF INHALATION OF 1-NP AND 2-NP ON RAT LIVER TOTAL GSH AND ON GSH-DEPENDENT ENZYME ACTIVITIES IN THE SOLUBLE FRACTION

| Day | Control | 1-NP | 2-NP |
|---|---|---|---|
| | a. Total GSH ($\mu$g/g liver) | | |
| 1 | 801 ± 97 | 706 ± 30 | 1004 ± 134 |
| 2 | 761 ± 41 | 750 ± 72 | 994 ± 201 |
| 4 | 759 ± 74 | 779 ± 41 | 1118 ± 90* |
| | b. GSH *S*-transferase (nmol CDNP conjugated/min/mg protein) | | |
| 1 | 908 ± 148 | 1156 ± 114 | 1259 ± 27* |
| 2 | 957 ± 63 | 1272 ± 201 | 1642 ± 219* |
| 4 | 953 ± 124 | 1023 ± 427 | 1399 ± 50* |
| | c. GSH peroxidase (nmol NADPH oxidized/mg protein) | | |
| 1 | 461 ± 63 | 613 ± 43* | 475 ± 85 |
| 2 | 489 ± 65 | 691 ± 20* | 491 ± 10 |
| 4 | 456 ± 34 | 549 ± 112 | 529 ± 77 |
| | d. GSSG reductase (nmol NADPH oxidized/min/mg protein) | | |
| 1 | 55.4 ± 4.8 | 50.6 ± 0.6 | 56.0 ± 2.3 |
| 2 | 58.1 ± 5.1 | 59.6 ± 11 | 55.1 ± 2.6 |
| 4 | 55.1 ± 2.8 | 46.1 ± 8.1 | 50.6 ± 2.3 |

* Significantly different from control at $P < 0.05$.

The higher degree of induction of GSH *S*-transferase activities by 2-NP compared to 1-NP corresponds to the fact that *in vitro* 2-NP is a better substrate for those isozymes than 1-NP (Habig *et al.*, 1974). The elevation of GSH *S*-transferase activities up to 72% and the unchanged levels of GSH peroxidase after exposure to 2-NP are in contrast to the findings of Zitting *et al.* (1981). Following the intraperitoneal injection of 50 mg/kg 2-NP, those authors observed levels of GSH *S*-transferase decreased by 16% and an induction of GSH peroxidase by 49% 24 hr after exposure. Conceivably, differences in the pharmacokinetic fate of 2-NP caused by the method of administration may be related to the observed differences in the response of GSH-dependent enzyme activities. A 250-g rat exposed for 7 hr to 100 ppm 2-NP inhales approximately 15 mg of the chemical based on a pulmonary ventilation rate of 100 ml/min. With a minimum absorption of 40% (Nolan *et al.*, 1982), the actual amount of 2-NP absorbed after 7 hr of exposure to 100 ppm 2-NP can be calculated to be about 6 mg or 24 mg/kg. Although the total amount of 2-NP absorbed by the animal may be comparable to the dose rate of 50 mg/kg used by Zitting *et al.* the different administration methods result in a different time course in the plasma level of the chemical: In a separate experiment it was observed that, with inhalation exposure to 100 ppm of 2-NP, plasma levels reach a plateau value of 1–3 $\mu$g/ml within 30 min after the start of the exposure (Haas-Jobelius, 1988). In contrast, Müller *et al.* (1983) demonstrated that, following injection of 25 mg/kg 2-NP, plasma levels attain peak concentrations of up to 20 $\mu$g/

ml shortly after administration and rapidly decline thereafter. This difference in the pharmacokinetics may conceivably also result in differences in the biological response of the organism.

## CONCLUSIONS

At an air concentration of 100 ppm, the overall effects on rat liver enzymes elicited by inhalation of the isomers 1-NP and 2-NP exhibit distinct differences. The effects on microsomal cytochrome P450 suggest that reactive intermediates may be formed in the metabolism of 2-NP, but not of 1-NP. This may be an indication of fundamental differences in the principal metabolism pathways of 1-NP and 2-NP in the rat.

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