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INDUCTION OF CYTOCHROME P450 ISOZYMES BY SIMULTANEOUS INHALATION EXPOSURE OF HENS TO *n*-HEXANE AND METHYL *iso*-BUTYL KETONE (M*i*BK)

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Abstract—Chickens were exposed simultaneously to the industrial hexacarbon solvents *n*-hexane and methyl *iso*-butyl ketone (M*i*BK). *n*-Hexane has been shown to be neurotoxic in both humans and other vertebrates. While M*i*BK is not neurotoxic, it has been shown to greatly synergize the clinical appearance of neurotoxicity in animals exposed to both of these solvents. Groups of hens were exposed for 29 days in inhalation chambers to 1000 ppm *n*-hexane in combination with 10, 100, 250, 500, or 1000 ppm M*i*BK. Other groups received either 1000 ppm *n*-hexane, 1000 ppm M*i*BK, or ambient air and served as controls. A dose-dependent decrease in body weight and an increase in clinical effects were noted for the highest exposure groups (1000 ppm *n*-hexane combined with 1000, 500 or 250 ppm M*i*BK). There was an M*i*BK dose-dependent increase in cytochrome P450 content and benzphetamine *N*-demethylase activity, but there was no distinct pattern for ethoxyresorufin *O*-deethylase or cytochrome *c* reductase activities. Mixed-function oxidase levels and activities (cytochrome P450 content and benzphetamine *N*-demethylase) were elevated significantly ($P < 0.05$) over controls even in the lowest M*i*BK group (10 ppm), although there were no clinical signs of neurotoxicity. Four different isozymes of cytochrome P450 were measured immunologically. There was a dose-dependent increase in three of the isozymes, two of which were phenobarbital inducible and one of which was induced by β-napthoflavone. Quantitatively, the largest increase was in the PB-A isozyme, a phenobarbital-inducible isozyme which accounted for approximately 70% of the cytochrome P450 present in animals treated with M*i*BK. The results suggest that M*i*BK selectively induces cytochrome P450 isozymes leading to the metabolic activation of the weak neurotoxicant *n*-hexane to the potent neurotoxicant 2,5-hexanedione (2,5-HD).

Aliphatic hexacarbons such as *n*-hexane, methyl *iso*-butyl ketone (M*i*BK), and methyl *n*-butyl ketone (M*n*BK) are used widely in industrial processes. Several grades of hexane are available, including 100% *n*-hexane as well as commercial grades which contain various amounts of *n*-hexane, *iso*-hexanes, and cyclopentanes [1]. Along with being prevalent in the workplace, M*i*BK has also become an environmental contaminant of concern due to improper or careless disposal practices [2]. The US threshold limit value (TLV-TWA) for exposure to *n*-hexane and M*i*BK vapors in the work place is 50 ppm [3].

The reason for concern regarding *n*-hexane exposure is due to reports that this solvent has been responsible for producing a central-peripheral distal neuropathy in industrial workers and "glue sniffers" [4, 5]. Similarly, M*n*BK has been implicated as the agent responsible for causing distal neuropathy in workers in a fabrics plant in Ohio [6]. Furthermore, laboratory experiments using a variety of animals (rats, cats, guinea pigs, and chickens) have indicated that both *n*-hexane and M*n*BK produce distal neuropathy resulting in ataxia which may progress to paralysis [5, 7–13]. Histologically, this neuropathy is characterized by giant axonal swellings and an accumulation of neurofilaments above the node of Ranvier. *n*-Hexane and M*n*BK are closely related, with M*n*BK being an oxidation product of *n*-hexane in biological and nonbiological systems. For both solvents, the final oxidation product, 2,5-hexanedione (2,5-HD), is thought to be the agent actually responsible for producing the neuropathy. On the other hand, despite reports of M*i*BK causing neuropathy in humans [14, 15], laboratory experiments with M*i*BK have failed to produce neuropathy in rodents, cats, or chickens [9, 12, 16]. In contrast to M*n*BK, M*i*BK is not an oxidation product of *n*-hexane metabolism, and cannot be metabolized to 2,5-HD.

Even though M*i*BK itself does not cause distal neuropathy, simultaneous inhalation of *n*-hexane and M*i*BK results in an increased, or synergized, neurotoxic effect over the toxicity of *n*-hexane alone [12]. Both solvents are commonly used industrially, so workers may be exposed to combinations of the two solvents. This paper reports the results of studies on the role of liver microsomal cytochrome P450 in the mechanism of the synergism of *n*-hexane neurotoxicity by M*i*BK.

MATERIALS AND METHODS

Animals

Young egg-laying hens (*Gallus gallus* domesticus) averaging 1.5 kg, purchased from Featherdown Farms (Raleigh, NC), were used for these experiments. These hens were both pathogen-free and chemical medication-free. The animals were acclimated to the inhalation chambers (see below)

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for 4–6 days prior to initiating the exposure experiments. They were provided with food (Layena Chicken Feed, Ralston Purina Co., St. Louis, MO) and water *ad lib.* during both the acclimation and experimental periods. Animals were weighed at the beginning of the experiment and on days 10, 20, and 29. They were observed for clinical effects daily.

Reagents

n-Hexane (99% pure) was obtained from Phillips Petroleum (Bartlesville, OK). ACS grade methyl *iso*-butyl ketone (4-methyl 2-pentanone; M*i*BK) was purchased from the Fisher Scientific Co. (Raleigh, NC). Radiolabeled *n*[1,2(5,6)-^{14}C]hexane (4.5 Ci/mmol) was obtained from Amersham, Inc. (Arlington Heights, IL). NADPH used for bioassays was purchased from the U.S. Biochemical Corp. (Cleveland, OH), while ethoxyresorufin was obtained from Molecular Probes (Junction City, OR). Benzphetamine-HCl was donated by The Upjohn Co. (Kalamazoo, MI). All other biochemicals were obtained from either the Sigma Chemical Co. (St. Louis, MO) or the Aldrich Chemical Co. (Milwaukee, WI).

Inhalation chambers

The animals were exposed in 11.4 ft^3 (0.32 m^3) stainless steel inhalation chambers (Young & Bertke Co., Cincinnati, OH) with controlled temperature and humidity conditions (21–24°, 40–60% humidity) which have been described in detail previously [10, 12]. In-chamber solvent concentrations were monitored daily using a Gow-Mac (Rahway, NJ) Series 750 gas chromatograph equipped with a 2 m × 2 mm i.d. glass column packed with Tenax GC 60/80 mesh and a flame ionization detector (FID). Vapor concentrations in the chambers varied by a maximum of 10%.

Inhalation exposure

Two similar 29-day inhalation exposure experiments were conducted in which chickens were exposed continuously to vapors of *n*-hexane, M*i*BK, or a combination of the two solvents. In the first experiment, five groups of animals consisting of five hens/group were exposed to 1000 ppm *n*-hexane, 1000 ppm M*i*BK, 1000 ppm *n*-hexane/1000 ppm M*i*BK, or 1000 ppm *n*-hexane/500 ppm M*i*BK; a control group was maintained in the Duke University Vivarium. In the second experiment, four groups of five animals each were exposed to 1000 ppm *n*-hexane in combination with 10, 100, and 250 ppm M*i*BK, along with a chamber control (air-exposed) group.

Enzyme assays

Chickens were killed by inhalation of carbon dioxide. Livers were perfused with 0.9% saline, and were then excised and homogenized in ice-cold 0.25 M sucrose/10 mM Tris–HCl/1 mM EDTA, pH 7.4. Microsomes were prepared using the calcium precipitation method [17]. The resulting microsomal pellet was washed with 150 mM KCl/10 mM Tris–HCl, pH 7.4, recentrifuged for 15 min, resuspended in this same buffer, divided into aliquots, and frozen at −70°.

Microsomal cytochrome P450 content was determined using the CO-reduced dithionite difference spectrum [18] using an extinction coefficient of 91 cm mmol on a Shimadzu (Kyoto, Japan) model UV-3000 spectrophotometer. Benzphetamine *N*-demethylase activity (BZND) was determined by incubating microsomes, substrate, and NADPH in 50 mM potassium phosphate buffer (pH 7.7) (total volume = 2 mL) for 15 min at 37°, and then stopping the reaction by adding 17% perchloric acid. Double strength Nash reagent was added to 1-mL aliquots and heated to 65° for 20 min to detect liberated formaldehyde by monitoring absorbance at 412 nM [19] using an LKB Ultraspec 4050 spectrophotometer (Cambridge, U.K.). Ethoxyresorufin *O*-deethylase activity (EROD) was determined according to Burke and Mayer [20] using a Perkin–Elmer (Norwalk, CT) LS-3 spectrofluorometer. Activity was determined in a 200 mM Tris–HCl buffer, pH 7.8, at 23° (total volume = 2 mL) by monitoring the increase in fluorescence due to resorufin at 1-min intervals for 3 min, using an excitation wavelength of 530 nm and an emission wavelength of 586 nm. Cytochrome *c* reductase activity was determined at 28° by measuring the change in absorbance at 550 nm in a 0.3 M KPO_4 buffer, pH 7.7, containing 0.5 mM cytochrome *c* (horse heart, type III). Absorbance was followed for 2 min to determine background change; then hen NADPH was added to initiate the reaction, which was followed for an additional 3 min. Cytochrome *c* reduction was determined using an extinction coefficient of 121 cm mmol [19]. Microsomal protein was determined according to Lowry *et al.* [21].

The *in vitro* metabolism of [^{14}C]*n*-hexane (15 or 30 μmol) was examined in liver microsomes (1 mg/mL) from animals treated with either 1000 ppm *n*-hexane or 1000 ppm M*i*BK. Following the incubation of [^{14}C]*n*-hexane with microsomes, 2-hexanone, γ-valerolactone, and 2,5-hexanedione were measured by HPLC as described previously [22].

Western blotting and quantitation of antibody binding to four isozymes of cytochrome P450

Two isozymes of cytochrome P450 (P450 *a* and *b*) were isolated from phenobarbital-treated hens and two from β-napthoflavone-treated hens and antibodies were produced in New Zealand white rabbits [23, 24]. Western blotting and dot-immunobinding were carried out as described previously [25]. Western blotting of microsomes was carried out following sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. Quantitation of these isozymes was assayed by the method of Jahn *et al.* [26]. Briefly, samples of microsomes were applied to nitrocellulose sheets using a slot-blot apparatus (Schleicher & Schuell, Keene, NH). Samples were diluted to contain 1% SDS, before diluting in radioimmunoassay (RIA) buffer (20 mM KCl, 120 mM NaCl, 2 mM $NaHCO_3$, 2 mM $MgCl_2$, 5 mM HEPES, pH 7.4, and 0.7% Triton X-100) to 0.5 mg/mL. Samples containing 10 μg of protein in 20 μL of buffer were loaded into each well. Antibodies were used at a dilution of 1:2000. Standard curves for each assay were constructed using purified cytochrome P450 isozymes. Antibody

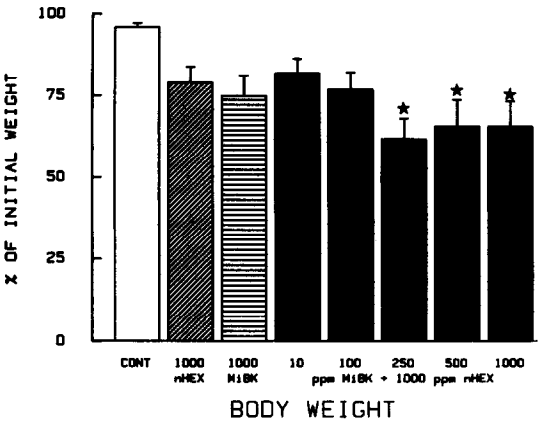

Fig. 1. Effects of *n*-hexane or M*i*BK or a combination of the two on body weight. Body weights of hens are expressed as a percent of initial body weight. Animals were treated, as described in Materials and Methods, by inhalation. The numbers of animals were N = 10 for the combined control groups and N = 5 for all other treatment groups except for the 1000 ppm *n*-hexane/500 ppm M*i*BK group where N = 4. Values are expressed as a percent of control (mean ± SE). Stars indicate a significant difference from control, $P < 0.05$.

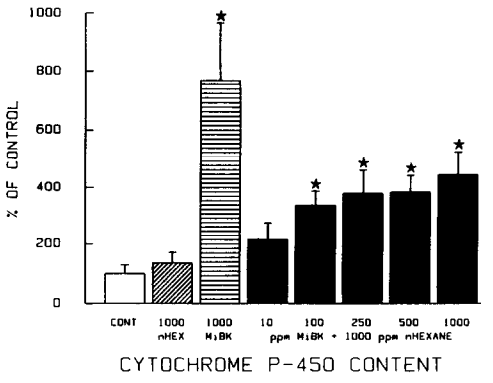

Fig. 2. Cytochrome P450 content of hens treated with *n*-hexane or M*i*BK or a combination of the two. The control (CONT) value was 0.108 nmol/mg protein. Values are expressed as a percent of control (mean ± SE). The numbers of animals were the same as in Fig. 1. Stars indicate a significant difference from control, $P < 0.05$.

reactivity was detected using ^{125}I-Protein-A [25] and quantified with a gamma counter (Packard Instruments).

Statistics

Data were analyzed using the analysis of variance portion (ANOVA) of the Statistical Analysis System (SAS) [27] on an IBM 3091 mainframe computer. When significant ANOVA F values were obtained, the data were analyzed further using the Scheffe option for multiple comparisons to determine which animal groups were significantly different at the 0.05% level.

RESULTS

Body weights

Overall, the animals exposed to combinations of *n*-hexane and M*i*BK lost weight in a dose-dependent manner, but there was considerable individual variability within each group (Fig. 1). The vivarium and chamber control were not significantly different from each other and were combined for comparative purposes in Fig. 1. Animals in all the other groups lost considerable weight during the first 10 days, slowed the rate of weight loss over the next 10 days, and then lost considerable amounts over the last 9 days. The 1000 *n*-hexane/1000 M*i*BK, 1000 *n*-hexane/500 M*i*BK, and 1000 *n*-hexane/250 M*i*BK groups lost significantly more weight (35–38%) than the controls. The remaining dose groups lost between 18 and 25% of their initial weight but, due to individual variability, were not significantly different from either the control or higher dose groups. One animal in the 1000 *n*-hexane/500 M*i*BK group died on day 23 after having lost 50% of its initial weight by day 20.

Observations

The animals showed M*i*BK dose-dependent signs of neurotoxicity, with clinical signs noticed in the three highest exposure groups (1000 ppm *n*-hexane and 1000, 500, and 250 ppm M*i*BK). The animals in the 250 ppm group showed mild ataxia, while those in the other two groups had moderate to severe ataxia (T3 to T4). These effects included a reluctance to walk, occasional stumbling, imbalance in gait, and difficulty in standing. One animal in the 1000 *n*-hexane/1000 M*i*BK group could not stand.

Gross examination of the livers did not reveal noticeable differences between the groups, except that the group receiving 1000 ppm M*i*BK alone appeared to have darker livers. Animals in all the exposure groups, including *n*-hexane alone, either completely stopped laying eggs within the first 5–7 days, or only rarely laid eggs at later times. Both the chamber controls and vivarium controls continued egg laying normally throughout the experiment.

Enzyme effects

Cytochrome P450 content. Hepatic microsomal cytochrome P450 content was induced in a dose-dependent manner in relation to M*i*BK exposure (Fig. 2). Both the vivarium and chamber control groups had low values (0.112 and 0.106 nmol/mg protein respectively) that were not significantly different and were combined for comparison to other groups. The 1000 ppm *n*-hexane group had a slightly, but not significantly, higher P450 content from the controls. P450 content in the remaining groups increased in proportion to M*i*BK exposure, ranging from 220% of the control value for the 1000 *n*-hexane/10 ppm M*i*BK exposure to 460% of the chamber control value in the group exposed to 1000 *n*-hexane/1000 ppm M*i*BK. Cytochrome P450 content in the group exposed only to 1000 ppm M*i*BK was 780% of the chamber control group. This group had significantly higher P450 content than any of the other exposure groups, including the 1000 *n*-hexane/1000 M*i*BK group. Cytochrome P450 in the groups

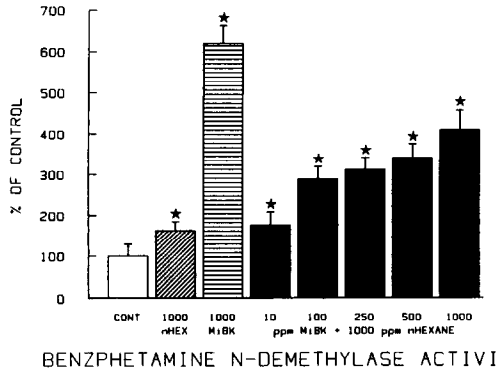

Fig. 3. Effects of *n*-hexane or M*i*BK or a combination of both on benzphetamine *N*-demethylase activity. BZND activity is expressed as a percent of control. Control value was 17 nmol HCOH formed/mg protein/15 min. Values are expressed as a percent of control (mean ± SE). Stars indicate a significant difference from control, $P < 0.05$. The numbers of animals were the same as in Fig. 1.

receiving 1000 ppm *n*-hexane and 1000, 500, 250, or 100 ppm M*i*BK all were significantly induced over the control animals. The 1000 *n*-hexane/10 M*i*BK group was not statistically significantly greater than the controls due to one hen that had a P450 content similar to the controls; excluding this hen, resulted in a significantly induced P450 content for this group. The Sorret peak of the CO reduced P450 complex in both control and M*i*BK induced organisms was 451 nm.

Benzphetamine N-*demethylase.* Microsomal benzphetamine *N*-demethylase (BZND) activity closely paralleled the pattern of cytochrome P450 content, with activity being induced in a dose-dependent manner relative to M*i*BK exposure (Fig. 3). Values for the two control groups were low (16 and 18 nmol HCOH/mg protein), and not significantly different and were combined for comparative purposes. The groups exposed to 1000 ppm *n*-hexane combined with 10, 100, 250, 500, or 1000 ppm M*i*BK had increasing activity with increasing M*i*BK exposure, ranging from a 190% increase for the 1000 *n*-hexane/10 M*i*BK group to 440% of the chamber control activity for the 1000 *n*-hexane/1000 M*i*BK group. All of these activities were significantly greater than control activities. Also, similar to the trend in P450 content, the greatest induction, 660% of control activity, was found in the group exposed only to 1000 ppm M*i*BK, and this activity was significantly higher than that of any other group. BZND activity in the group exposed only to 1000 ppm *n*-hexane was just barely significantly greater than activity in the chamber control group, but not significantly greater than activity in the vivarium controls. The increases in BZND activity found with M*i*BK exposure were similar to, but slightly smaller than, the increases in cytochrome P450 content for similar exposures.

In vitro *metabolism of* [^{14}C]n-*hexane.* There was a significant increase in the *in vitro* metabolism of *n*-hexane to 2,5-hexanedione in hens treated with 1000 ppm M*i*BK as compared to those treated with 1000 ppm *n*-hexane (Table 1). In addition, there was also an increased formation of γ-valerolactone in M*i*BK-treated animals.

Ethoxyresorufin O-*deethylase and cytochrome* c *reductase.* There was no discernible, dose-related pattern for either ethoxyresorufin *O*-deethylase or cytochrome *c* reductase activities in these studies.

Isozymes of cytochrome P-450. Three out of four isozymes of cytochrome P450 (PB-A, PB-B and β-NF-C) exhibited a clear dose–response relation to M*i*BK concentration (Fig. 4). In all cases control groups were not significantly different from each other and were combined for comparison with treated groups. In general, M*i*BK alone exhibited the greatest induction in cytochrome P450 (from 200 to 750% of control), with groups receiving both M*i*BK and *n*-hexane demonstrating lesser degrees of induction. Treatment with *n*-hexane alone resulted in a modest increase in all isozymes of cytochrome P450. The greatest percent induction was found with the PB-A isozyme. This isozyme was also quantitatively present in the greatest amount, accounting for greater than 70% of the cytochrome P450 present. Figure 5 illustrates a Western blot of PB-A cytochrome P450 showing the increase in this isozyme, after treatment with either 500 or 1000 ppm M*i*BK and 1000 ppm *n*-hexane, that was significantly different with dot-immunobinding. These data were used to confirm the induction of specific cytochromes P450 found by dot-immunobinding.

DISCUSSION

This work represents a study into the mechanisms of the synergistic effect of M*i*BK on *n*-hexane neuropathy that has been reported previously [12]. Although focusing on joint neurotoxic action in chickens simultaneously exposed to 1000 ppm M*i*BK and 1000 ppm *n*-hexane, Abou-Donia *et al.* [12] also reported that an induction of cytochrome P450 occurred with exposure to 1000 ppm M*i*BK. In the present study, we further investigated the mechanism of this synergism by identifying specific isozymes of cytochrome P450 which were induced by the joint action of *n*-hexane and M*i*BK.

Dose–response related cytochrome P450 effects were studied by exposing chickens to 1000 ppm M*i*BK and 1000 ppm *n*-hexane alone as well as with doses of 10, 100, 250, 500, and 1000 ppm M*i*BK in combination with *n*-hexane. In addition to determining P450 content, several mixed-function oxidase-related enzymatic activities were also determined, including BZND [19] and EROD [20]. The results indicated a dose-dependent increase in cytochrome P450 content with increasing M*i*BK exposure, and an almost parallel induction of BZND activity (Figs. 2 and 3). In contrast, there was no distinct dose-related pattern for EROD activity. Furthermore, the Sorret peak of the CO-reduced P450 complex was 451 nm for both induced and control animals, rather than shifting downward as is commonly found with P448 induction.

Antibodies to specific isozymes of cytochrome P450 revealed that two phenobarbital-inducible

Table 1. *In vitro* metabolism of [^{14}C]*n*-hexane by chicken hepatic microsomes

Substrate	Product	1000 ppm *n*-hexane microsomes	1000 ppm MiBK microsomes	% of 1000 ppm hexane microsomes
15 μmol				
n-Hexane	2-Hexanone	96.0 ± 5.5	80.3 ± 0.8	83.6
	γ-Valerolactone	34.5 ± 1.5	43.0 ± 0.7*	125
	2,5-HD	26.7 ± 1.2	55.5 ± 8.0*	208
30 μmol				
n-Hexane	2-Hexanone	110.0 ± 8.0	103.0 ± 2.5	93.6
	γ-Valerolactone	35.4 ± 0.6	91.5 ± 1.5*	258
	2,5-HD	42.5 ± 5.0	73.0 ± 2.5*	172

Values, expressed in nmol product/hr, are means ± SD, N = 5.
* Significantly different from microsomes of hens treated with 1000 ppm *n*-hexane, $P < 0.05$.

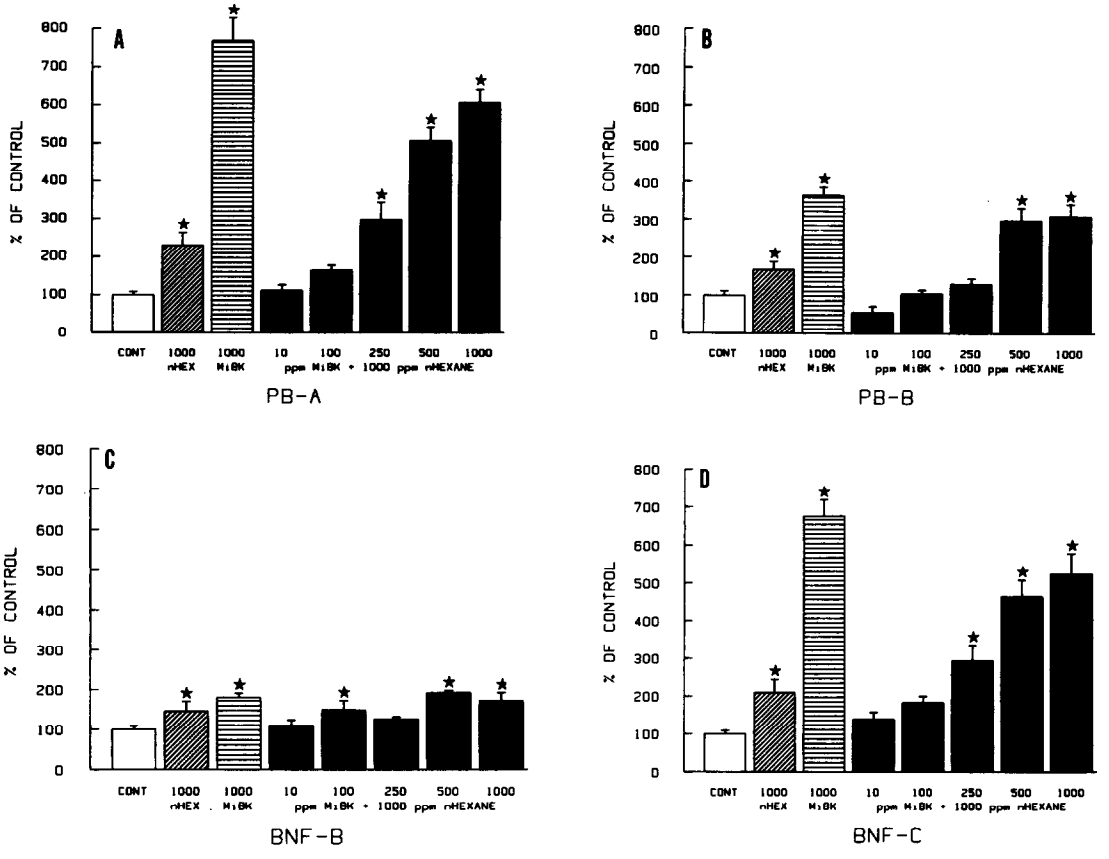

Fig. 4. Dot-immunobinding of four different isozymes of cytochrome P450, (A) PB-A, (B) PB-B, (C) βNF-B, and (D) βBNF-C, expressed as a percent of control. Control values (in nmol/mg protein) were: PB-A, 0.0767; PB-B, 0.0458; βNF-B, 0.0054; and βNF-C, 0.013. Values are expressed as a percent of control (mean ± SE). Stars indicate a significant difference from control, $P < 0.05$. The numbers of animals were the same as in Fig. 1.

isozymes were increased significantly in a dose-dependent manner. In addition, one β-naphthoflavone-inducible isozyme was also increased; however, this isozyme made up a small amount of the total cytochrome P450. The PB-A isozyme showed the greatest increase, in both total amount as well as in percent induction compared to control.

The dose-related increase in cytochrome P450 content, BZND activity and specific cytochrome P450 isozymes with increasing MiBK exposure complements the increased neurotoxicity index reported for exposure to 100, 250, 500, and 1000 ppm MiBK in combination with 1000 ppm *n*-hexane [12]. Other reports have indicated that *n*-hexane is a relatively weak neurotoxicant that is metabolized by the monooxygenase system to 2-hexanol [28], which

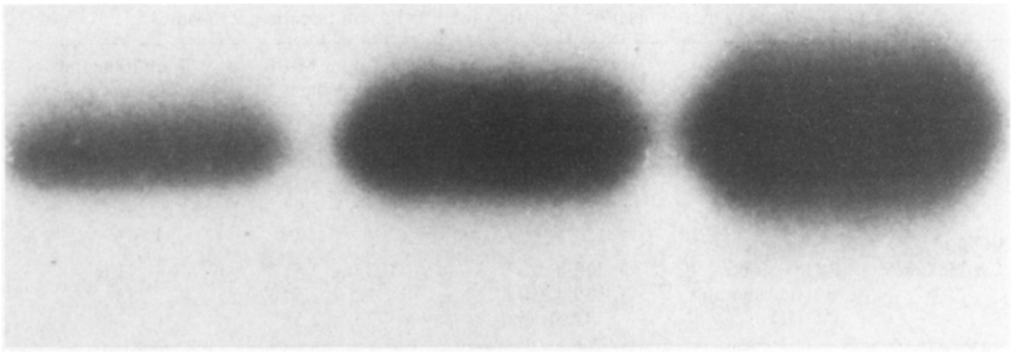

Fig. 5. Western blot of PB-A cytochrome P450 showing the increase in this isozyme after treatment with either 500 or 1000 ppm *M*iBK and 1000 ppm *n*-hexane. Cont. is control.

is then further oxidized to 2-hexanone (M*n*BK), 2,5-hexanediol, 2-hydroxy-5-hexanone, and 2,5-HD [7, 8, 29]. 2,5-HD is regarded as being responsible for the observed neurotoxic effects [11, 29]. The observed dose-related increases in both cytochrome P450 and the neurotoxicity index suggest that the synergistic effect of M*i*BK or *n*-hexane neurotoxicity may be due to increased oxidative metabolism of *n*-hexane by induced microsomal enzymes. *In vitro* metabolism using hepatic microsomes from M*i*BK-exposed animals and *n*-hexane-exposed animals showed an increased formation of 2,5-HD from *n*-hexane by microsomes from M*i*BK-exposed animals. Also, other reports have indicated that rats are more susceptible than chickens to *n*-hexane neuropathy possibly due to the normally lower cytochrome P450 content and enzymatic activity of chicken microsomes [30, 31]. Although we do not know if mammalian cytochrome P450 is induced by M*i*BK, studies by other investigators have demonstrated that cytochrome P450IIE1 and P450IIB1 were induced in rats by 2-hexanone [32].

These studies indicate that the potentiation of *n*-hexane neurotoxicity by M*i*BK is most likely due to the induction of specific isozymes of cytochrome P450. The majority of the increase in P450 is in the phenobarbital-inducible isozymes; however, there was also an increase in at least one β-napthoflavone-inducible isozyme. The induction of cytochrome P450 then leads to an increased metabolism of *n*-hexane to more reactive metabolites leading to enhanced neurotoxicity.

Interestingly, while M*i*BK synergizes the neurotoxic effects of *n*-hexane, the greatest induction of cytochrome P450 and BZND activity occurred in animals exposed solely to 1000 ppm M*i*BK. Thus, 1000 ppm M*i*BK exposure resulted in significantly greater induction of these microsomal indices than exposure to 1000 ppm *n*-hexane/1000 ppm M*i*BK, suggesting that simultaneous *n*-hexane exposure interferes with M*i*BK microsomal induction in some manner. Currently, we can only speculate that this effect may be associated with either delivery to or uptake of M*i*BK by liver tissue, or else is due to competition with concurrent metabolism of *n*-hexane in the liver. Exposure to 1000 ppm *n*-hexane alone resulted in a slight increase in cytochrome P450.

A further goal of this study was to determine if a threshold exposure or no effect dose for M*i*BK induction of cytochrome P450 exists. We found significant induction in cytochrome P450 content and BZND activity at the lowest M*i*BK concentration tested, 10 ppm, so any no-effect dose would be below this exposure. In terms of occupational safety, this suggest that chronic inhalation of even low concentrations of M*i*BK could increase the risk of *n*-hexane neuropathy in workers exposed to both solvents, and is particularly important when considering that some 436,000 workers may be exposed to M*i*BK [33].

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