Biochemical Pharmacology, Vol. 38, No. 20, pp. 3461–3467, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

EFFECTS OF ACETONE AND METHYL *n*-BUTYL KETONE ON HEPATIC MIXED-FUNCTION OXIDASE*

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(*Received* 7 *October* 1988; *accepted* 24 *February* 1989)

Abstract—The administration of ketones potentiates CCl_4 hepatotoxicity; however, the potencies of the ketones differ. The aim of the present study was to assess potential differences between acetone and methyl *n*-butyl ketone (MnBK) on cytochrome P-450. The effects of single and repetitive doses of acetone and MnBK were determined in male rats by estimating the rate of metabolite formation of three substrates and the hepatic content of cytochrome P-450. A single treatment with acetone (13.5 mmol/kg or greater) enhanced the oxidation of aniline and 7-ethoxycoumarin, whereas repetitive treatments also increased aminopyrine demethylation and cytochrome P-450 content. Single and repetitive treatments of MnBK (15 mmol/kg) augmented the oxidation of all three substrates and increased cytochrome P-450 content. The effects of the ketones on cytochrome P-450 isozymes were characterized using sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Acetone and MnBK increased the 52.1 and 54.1 kD forms and, in addition, MnBK tended to increase the 50.6 kD species. The data indicate that ketones differ in the type of isozymes induced and in the degree of induction. The higher potency of MnBK, compared to acetone, is probably associated with the fact that MnBK affects a greater number of isozymes than acetone.

Two decades ago it was demonstrated that isopropanol potentiates the hepatotoxicity of carbon tetrachloride (CCl_4) in the rat [1, 2]. It was also observed that ethanol and other aliphatic alcohols exacerbate the liver injury produced by various haloalkanes [3–6]. The potentiating capacities of the alcohols differ significantly; isopropanol is more potent than the others [2]. Isopropanol potentiation of CCl_4 hepato- and nephrotoxicity has also been shown to occur in humans [7, 8].

Evidence indicates that the potentiating activity of isopropanol is due to the production of a ketonic metabolite, acetone [9, 10]. Other ketones are capable of potentiating haloalkane hepatoxicity [11]. Their potentiating abilities appear to be variable; equimolar doses of methyl *n*-butyl ketone are more potent than those of 2,5-hexanedione or acetone [12].

The ability of haloalkanes to produce hepatoxicity depends on a sensitive balance between detoxification mechanisms and the generation of reactive metabolites. The latter process is closely associated with the activity of hepatic mixed-function oxidase (MFO), as the toxicity of haloalkanes is secondary to the formation of reactive metabolites [13]. For instance, the toxicity of chloroform depends on the production of phosgene [14], and the toxicity of CCl_4 is related to the formation of an electrophilic chlorine [14] by the hepatic MFO.

Ketones can enhance the activity of liver cytochrome P-450 [14–17], and their abilities to potentiate haloalkane hepatotoxicity appear to be related to their capacities to induce cytochrome P-450. The objective of the present study was to assess why methyl *n*-butyl ketone has a greater ability than acetone to potentiate haloalkane hepatotoxicity. We studied the effects of acetone and methyl *n*-butyl ketone on the activity of total cytochrome P-450 and on the relative amounts of selected isozymes of this cytochrome.

METHODS

Animals. Healthy male rats of the Sprague–Dawley strain weighing 250–350 g, purchased from Charles River Canada Inc. (St Constant, Québec), were used throughout the study. The animals were maintained on Purina Lab Chow (No. 4020) and water *ad lib.* for at least 1 week before initiation of experiments and had access to food and water until the moment they were killed.

Experimental protocol. Acetone (A & C, American Chemicals Ltd., Montréal, Québec) was given to groups of six to eight rats by gavage in a solution of water, at the following single doses: 1.4, 2.7, 6.8, 13.5, 27.1 or 54.1 mmol/kg. Three other groups of six to eight rats received 1.4, 2.7 or 13.5 mmol/kg of acetone every 24 hr for 5 days. In all cases the final volume administered was 10 ml/kg. The control group (N = 10) received 10 ml/kg of water. All animals were killed by decapitation 24 hr after the last dose of acetone.

Methyl *n*-butyl ketone (MnBK) (Aldrich Chemical Co, Milwaukee, WI) was administered by gavage in a solution of corn oil. Groups of six to eight rats

* This work was partially supported by the NSERC (Strategic Grants Program).

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received single doses of 1.5, 3.0, 5.0, 15.0 or 30.0 mmol/kg. Two other groups of six to eight rats received 1.5 or 5.0 mmol/kg of MnBK every 24 hr for 5 days. In all cases the final volume administered was 10 ml/kg. Control rats (N = 10) received 10 ml/kg of corn oil. The rats were killed by decapitation 24 hr after the last dose of MnBK.

Preparation of microsomes. After decapitation, the livers were immediately excised, perfused with a solution of 1.5% KCl (w/v), excised, and blotted dry. Liver homogenates (20%) were prepared in ice-cold sucrose (0.25 M) and centrifuged at 600 *g* for 10 min. The supernatant fraction was centrifuged at 12,000 *g* for 10 min, and to each 10 ml of the resulting supernatant fraction 0.1 ml of $CaCl_2$ (1.0 M) was added [18]. Following centrifugation at 27,000 *g* for 15 min, the pellet was resuspended in 1.15% KCl (w/v) and centrifuged at 27,000 *g* for 15 min. All the above operations were performed at 4°. The resulting pellet was covered with sucrose (0.25 M) and kept frozen at −40°.

Assays. Protein content in the microsomal preparation was determined by spectrophotometry [19]. Aminopyrine N-demethylation was assessed by measuring the rate of formation of formaldehyde using a colorimetric method [20]. The O-dealkylation of 7-ethoxycoumarin was evaluated by determining the rate of the formation of 7-hydroxycoumarin using a fluorimetric method [21]. The hydroxylation of aniline was assessed by measuring the rate of formation of *p*-aminophenol, using a colorimetric method [22]. Total cytochrome P-450 was determined by optical difference spectrophotometry [23].

The effects of acetone and MnBK on hepatic MFO were further characterized by assessing ketone-induced relative changes of several cytochrome P-450 isozymes. Three groups of six rats each received by gavage the vehicle, acetone (27.1 mmol/kg), or MnBK (30 mmol/kg). These doses were selected because they were fully effective for increasing the activity of cytochrome P-450. The rats were killed by decapitation 24 hr after the treatment and hepatic microsomes were isolated as described above. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of solubilized microsomal preparations was carried out using the method described by Laemmli [24] with minor modifications. Briefly, the stacking gel consisted of 4.87% (w/v) acrylamide and 0.13% (w/v) *N,N'*-methylene-bis-acrylamide, a gel buffer of 125 mM Tris–HCl, 0.1% (w/v) sodium dodecyl sulfate, 0.1% (w/v) ammonium persulfate and 0.05% (w/v) tetramethylenediamine at pH 6.8. The separating gel was 12 cm long and contained 7.3% acrylamide, 0.2% *N,N'*-methylene-bis acrylamide, 0.015% (w/v) ammonium persulfate, 0.13% tetramethylenediamine with a buffer gel [0.375 M Tris–HCl and 0.1% (w/v) sodium dodecyl sulfate], and 0.025% (w/v) tetramethylenediamine at pH 8.8. Microsomal preparations were solubilized in 62.5 mM Tris–HCl (pH 6.8), 5% (w/v) β-mercaptoethanol, 3% (w/v) sodium dodecyl sulfate, 5% (w/v) of a solution of 0.25% blue bromophenol and 10% glycerol.

Six microliters of the solubilized microsomal preparation was applied to each gel, and the electrophoresis (LKB 2301 power supply, LKB, Bromma, Sweden) was carried out using varying currents: 20 mA for 2 hr, 3 mA overnight and 40 mA until the colored front had migrated to the bottom of the gel. The solution for electrophoresis was composed of 192 mM glycine, 25 mM Tris and 0.1% (w/v) sodium dodecyl sulfate.

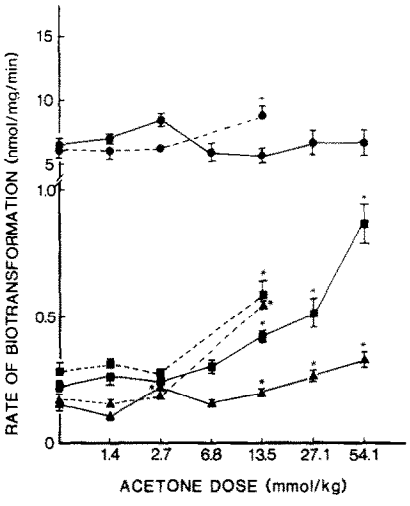

Fig. 1. Rate of biotransformation of 7-ethoxycoumarin (▲), aniline (■) and aminopyrine (●) following single doses (———) or daily doses for 5 days (– – –) of acetone. Vertical bars represent SE. Key: (*) $P < 0.05$ vs control (dose 0).

The gel was rinsed twice in the solution used to transfer the proteins on nitrocellulose, containing 25 mM Tris base, 192 mM glycine and 20% methanol at pH 8.3. The transfer was done using the Transfor Electroblotting Unit (LKB) on 0.45 μm pore size nitrocellulose membranes, applying 0.3 A for 2.5 hr. Thereafter, the nitrocellulose membranes were rinsed with a solution containing 10 mM Tris and NaCl (150 mM) at pH 7.4. Finally, the nitrocellulose membrane and the gel were colored with Fast Green and Coomassie brilliant blue dyes respectively. The molecular weight of cytochrome P-450 isozymes was determined by comparing their length of migration to the migration of proteins with a known weight. The nitrocellulose membranes were scanned at 550 nm with an LKB densitometer (LKB), after two successive decolorations in 95% ethanol, glacial acetic acid and water (25/10/65).

Statistical analysis. The comparison of effects of the various doses of acetone and MnBK to the control group was carried out using a one-way analysis of variance for parallel groups. The statistical difference was determined using the Dunnett's distribution table [25]. The level of significance was established at $P < 0.05$.

RESULTS

Effect of acetone on the oxidation of aminopyrine, 7-ethoxycoumarin and aniline and on cytochrome P-450 total content. When acetone was administered as a single oral dose, the rate of aminopyrine demethylation was not affected (Fig. 1). The rates of 7-ethoxycoumarin O-dealkylation and aniline

Table 1. Cytochrome P-450 content determined following single doses or daily doses of acetone

Acetone	Cytochrome P-450 content (nmol/mg protein)
Single dose (mmol/kg)	
Control	0.68 ± 0.06
1.4	0.72 ± 0.07
2.7	0.94 ± 0.08
6.8	0.64 ± 0.07
13.5	0.70 ± 0.06
27.1	0.89 ± 0.13
54.1	0.89 ± 0.29
Daily (5 days) dose (mmol/kg/day)	
Control	0.70 ± 0.06
1.4	0.71 ± 0.04
2.7	0.77 ± 0.07
13.5	0.95 ± 0.07*

Values are means ± SE, N = 6–8.
* $P < 0.05$ compared to the respective control.

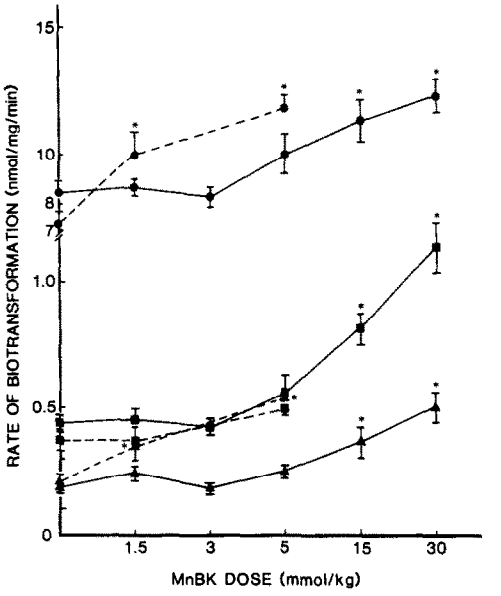

Fig. 2. Rate of biotransformation of 7-ethoxycoumarin (▲), aniline (■) and aminopyrine (●) following single doses (———) or daily doses for 5 days (– – –) of methyl *n*-butyl ketone (MnBK). Vertical bars represent SE. Key: (*) $P < 0.05$ vs control (dose 0).

hydroxylation were increased following 13.5 mmol/kg or more of acetone. Cytochrome P-450 total content measured in the microsomal fraction was not affected by single doses of acetone (Table 1).

The daily administration of acetone for 5 days increased the rates of aminopyrine demethylation, 7-ethoxycoumarin O-dealkylation and aniline hydroxylation, only at a dose of 13.5 mmol/kg/day (Fig. 1). On the other hand, repetitive treatment with acetone was more effective than single doses for increasing 7-ethoxycoumarin O-dealkylation. Repetitive treatment with 13.5 mmol/kg/day appeared to be more effective than 4-fold higher single dose (54.1 mmol/kg) ($P < 0.05$). The amount of cytochrome P-450 was increased only after the repetitive administration of 13.5 mmol/kg of acetone (Table 1).

Effect of MnBK on the oxidation of aminopyrine, 7-ethoxycoumarin and aniline and on cytochrome P-450 total content. The administration of MnBK, as a single oral dose, enhanced the oxidation of the three substrates used, at doses of 15 mmol/kg or greater (Fig. 2). MnBK administered at a dose of 15 mmol/kg or more also increased the total amount of cytochrome P-450 (Table 2).

The administration of MnBK daily for 5 days appeared to have an additive effect on the rate of 7-ethoxycoumarin O-dealkylation and on the demethylation of aminopyrine (Fig. 2). Interestingly, multiple treatments of MnBK modified the hydroxylation of aniline to an extent similar to that of a single dose. Repetitive treatments with MnBK (5 mmol/kg/day) increased the amount of total cytochrome P-450 to the same degree as did a single treatment of 30 mmol/kg, suggesting an additive effect.

Effects of acetone and MnBK on cytochrome P-450 isozymes. As shown in the SDS–polyacrylamide slab gels (Fig. 3), when judged by the intensity of protein-staining bands in microsomes, acetone or MnBK only induced the band with a molecular

Table 2. Cytochrome P-450 content determined following single doses or daily doses of MnBK

MnBK	Cytochrome P-450 content (nmol/mg protein)
Single dose (mmol/kg)	
Control	0.84 ± 0.05
1.5	1.11 ± 0.17
3	1.04 ± 0.06
5	0.94 ± 0.05
15	1.34 ± 0.13*
30	1.52 ± 0.12*
Daily (5 days) dose (mmol/kg/day)	
Control	0.94 ± 0.06
1.5	0.94 ± 0.03
5.0	1.51 ± 0.10*

Values are means ± SE, N = 6–8.
* $P < 0.05$ compared to the respective control.

weight of approximately 52.1 kD, corresponding to the form P-450$_d$. On the other hand, when comparing the densitometric scans (Fig. 4 and Table 3), it is apparent that MnBK tended to produce a small induction ($P < 0.1$) of the isozyme with a molecular weight of 50.6 kD. The administration of acetone did not influence this isozyme. Both ketones induced the isozyme corresponding to P-450$_d$. In addition, both ketones induced the isozyme with a molecular weight of about 54.1 kD. If we keep in mind that the doses of the ketones administered were similar, MnBK appears to be more potent than acetone according to the densitometric scan. Other isozymes

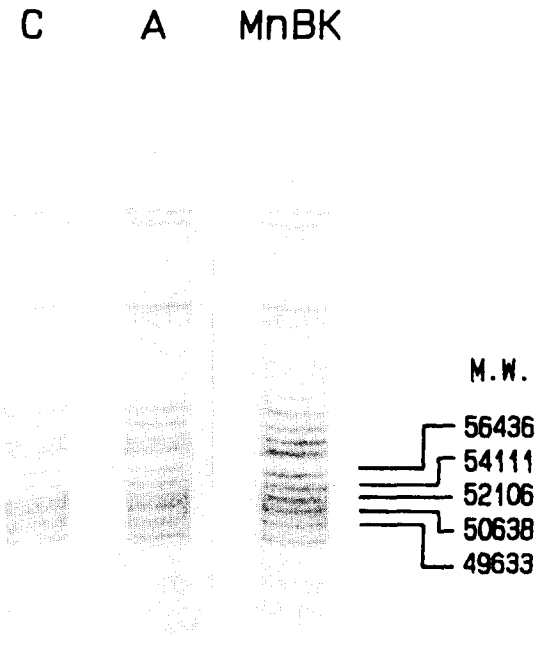

Fig. 3. SDS–polyacrylamide slab gel of hepatic microsomes from control rats (C) and rats receiving acetone (A) or methyl *n*-butyl ketone (MnBK).

possibly related with the biotransformation of xenobiotics were not affected.

DISCUSSION

Acetone, when administered as a single dose, did not influence the amount of total cytochrome P-450 nor the rate of aminopyrine demethylation, independently of the dose administered. Doses of 13.5 mmol/kg or greater, however, increased the rate of 7-ethoxycoumarin O-dealkylation and of aniline hydroxylation. These results are in agreement with those reported by others using isopropanol, a solvent generating acetone [15, 17]. On the other hand, MnBK increased the amount of total cytochrome P-450, as well as the rate of oxidation of the three substrates used.

Single doses of acetone or MnBK enhanced aniline hydroxylation to a similar extent. After 13.5 and 27.1 mmol acetone/kg, the rate of aniline hydroxylation increased by 90 and 130% respectively; following 15 and 30 mmol/kg of MnBK, it increased by 84 and 156% respectively. In contrast, a single dose of MnBK appeared to be about three times more effective than acetone for increasing the rate of 7-ethoxycoumarin O-dealkylation. After 15 mmol/kg of MnBK, the O-dealkylation of 7-ethoxycoumarin increased 103% compared to 29% following 13.5 mmol/kg of acetone; after 30 mmol/kg of MnBK it increased 178% compared to 66% following 27.1 mmol/kg of acetone. Thus, in some respects, the two ketones differ qualitatively and quantitatively, while they also possess some common properties.

Acetone induced the P-450 isozymes with molecular weights of 52.1 and 54.1 kD and decreased the amount of the 59.5 kD isozyme (Table 3). On the other hand, MnBK tended to increase the isozyme with 50.6 kD and increased significantly the 52.1 and 54.1 kD isozymes. When the effect of acetone on cytochrome P-450 isozymes was compared to that produced by MnBK, it was clear that the latter produced a greater induction of these isozymes.

It is interesting to note that there is parallelism between the changes induced by the ketones in the rate of oxidation of aminopyrine, aniline and 7-ethoxycoumarin and the changes in the various P-450 isozymes. Effectively, aminopyrine is preferentially demethylated by P-450$_b$, P-450$_a$ and P-450$_e$, with molecular weights ranging from 48 to 52 kD; these forms are preferentially induced by phenobarbital [26–28]. We assume that in our experiments the 50.6 kD isozyme corresponds to one of the forms induced by phenobarbital. Acetone did not modify the rate of demethylation of aminopyrine, nor the relative amount of the 50.6 kD form. On the other hand, MnBK enhanced the rate of demethylation of aminopyrine and tended to increase the 50.6 kD isozyme. Aniline appears to be hydroxylated by isozyme P-450$_d$, a form with a molecular weight of 52 kD [26], which probably corresponds to the 52.1 kD form seen in our experiments. Acetone and MnBK increased both the rate of hydroxylation of aniline and the relative amount of the isozyme with a molecular weight of 52.1 kD.

Concerning the dealkylation of 7-ethoxycoumarin, it is more difficult to specify the isozyme(s) involved. The dealkylation of 7-ethoxycoumarin appears to be rather specifically carried out by the species induced by 3-methylcholanthrene (3MC). In our hands, 3MC induced two isozymes of molecular weights of 52.6 and 55.6 kD, which were not detectable in rats

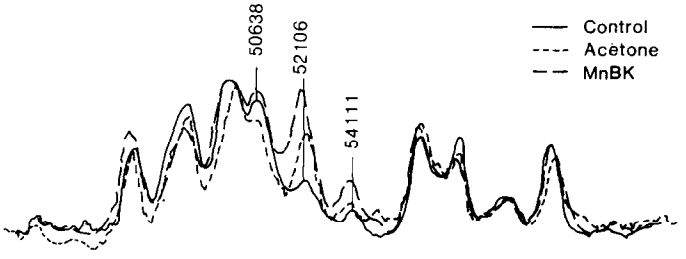

Fig. 4. Densitometric scans at 550 nm of the Fast Green stained proteins from control rats, and rats receiving acetone or methyl *n*-butyl ketone.

Table 3. Intensities of hepatic cytochrome P-450 isozymes, as determined by scan densitometry, in rats that received a single dose of the vehicle, acetone or MnBK

	Absorbance									
	Molecular weight (kD)									
	46.6	48.2	49.6	50.6	52.1	54.1	56.4	57.8	59.5	61.5
Control (vehicle)	61 ± 12	91 ± 6	129 ± 11	122 ± 16	47 ± 3	19 ± 1	63 ± 8	50 ± 3	45 ± 7	53 ± 3
Acetone (27.1 mmol/kg)	66 ± 10	101 ± 9	119 ± 17	126 ± 16	84 ± 12*	28 ± 4*	56 ± 8	44 ± 6	27 ± 2*	51 ± 3
MnBK (30 mmol/kg)	64 ± 7	94 ± 7	132 ± 7	160 ± 24	102 ± 7*	44 ± 4*	54 ± 10	46 ± 4	35 ± 3	54 ± 2

Values are means ± SE, N = 6–8.
* $P < 0.05$ compared to the respective control.

receiving the vehicle or the ketones (unpublished results). Therefore, keeping in mind that MnBK was a more potent activator of the dealkylation of 7-ethoxycoumarin than acetone, and that MnBK induced the 54.1 kD isozyme more than acetone, we may speculate that in rats, not induced by 3MC, the isozyme dealkylating 7-ethoxycoumarin has a molecular weight of 54.1 kD. Such speculation is not unreasonable, since for theophylline the isozymes involved in its biotransformation differ if the animals are induced with phenobarbital or 3MC [29]. If our assumption is true, we may state that the effects of acetone and MnBK on the rate of dealkylation of 7-ethoxycoumarin parallel those produced on the 54.1 kD isozyme.

It has been reported that pretreatment of rats with 3MC does not potentiate CCl_4 hepatotoxicity [30–32]. On the other hand, it is well accepted that 3MC is a potent inducer of the O-dealkylation of 7-ethoxycoumarin. Our results suggest that potentiation of CCl_4 hepatotoxicity is essentially associated with the induction of the 52.1 kD isozyme. Both acetone and MnBK increase the rate of aniline hydroxylation and both are able to increase the rate of production of reactive metabolites of chloroform and of CCl_4 [33, 34]. In addition, it has been shown in a reconstituted system that a 52-kD cytochrome P-450 is necessary to generate the active trichloromethyl radical (CCl_3) from CCl_4 [35, 36].

The difference between the abilities of acetone and MnBK to potentiate hepatotoxicity may be associated with the fact that MnBK tended ($P < 0.1$) to enhance the 50.6 kD isozyme, as reflected by an increase in the rate of aminopyrine demethylation and in the total amount of cytochrome P-450. It is presently known that the biotransformation of CCl_4 generates several reactive metabolites, such as the trichloromethyl radical, the trichloromethyl carbanion, the electrophilic form of chlorine, and phosgene [13, 37]. Some of these reactive metabolites may be produced by the 50.6 kD isozyme. Supporting this hypothesis is the fact that pretreatment with phenobarbital, known to potentiate CCl_4 hepatotoxicity [38], induces several isozymes with molecular weights ranging from 48 to 52 kD [26–28] and, as a consequence, increases the total amount of cytochrome P-450.

Thus, we believe that there are several forms of cytochrome P-450 which may be induced and consequently enhance CCl_4 hepatotoxicity. These forms may be those that are primarily associated with aniline hydroxylase and aminopyrine demethylase activities. Consistent with this hypothesis is the observation that aminotriazol, an hepatic catalase inhibitor, inhibits the activity of aniline hydroxylase; however, it does not prevent completely isopropanol potentiation of CCl_4 hepatotoxicity [39]. In the present study repetitive administration of acetone did not alter aniline hydroxylase activity but increased considerably aminopyrine demethylase activity, as well as the total amount of cytochrome P-450. On the other hand, repetitive administration of acetone appears to be additive in potentiating CCl_4 hepatotoxicity [40]. Therefore, forms of cytochrome P-450 other than the one responsible for

aniline hydroxylation appear likely to be involved in CCl_4 potentiation.

In conclusion, our results suggest that more than one form of cytochrome P-450 is implicated in the potentiation of haloalkane hepatotoxicity. The plurality of forms involved may explain the differences observed when using acetone or MnBK to potentiate haloalkane hepatotoxicity.

Acknowledgement—The authors thank Mrs Lise Jutras for her excellent technical assistance.

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