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FUNDAMENTAL AND APPLIED TOXICOLOGY **10**, 431–438 (1988)

Assessment of the Minimal Effective Dose of Acetone for Potentiation of the Hepatotoxicity Induced by Trichloroethylene–Carbon Tetrachloride Mixtures[1]

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Assessment of the Minimal Effective Dose of Acetone for Potentiation of the Hepatotoxicity Induced by Trichloroethylene–Carbon Tetrachloride Mixtures. CHARBONNEAU, M., PERREAULT, F., GRESELIN, E., BRODEUR, J., AND PLAA, G. L. (1988). *Fundam. Appl. Toxicol.* **10**, 431–438. Administration of acetone to rats in amounts larger than or equal to a minimal effective dosage (MED) is known to potentiate the severity of the liver damage produced by CCl_4 alone. It has been reported that CCl_4-induced hepatotoxicity is also enhanced by the previous administration of trichloroethylene (TCE). In addition, TCE–CCl_4 mixture-induced liver injury is potentiated by acetone. The present study was undertaken to determine if the acetone MED is decreased when the haloalkane challenge is a mixture of TCE–CCl_4 instead of CCl_4 alone. The effect of varying mixture compositions was also evaluated. In a first series of experiments, male Sprague–Dawley rats received corn oil or acetone (0.05–0.25 ml/kg, po); 18 hr later, they received an ip injection of either CCl_4 (0.1 ml/kg) or [TCE (0.25 ml/kg)–CCl_4 (0.1 ml/kg)]. In a second series, rats received corn oil or acetone (0.75 ml/kg), and were challenged with TCE (1.5 ml/kg), CCl_4 (0.25 ml/kg), or a mixture of TCE–CCl_4, where TCE and CCl_4 dosages were equal to 25–75%, 50–50%, and 75–25%, respectively, of those used for the administration of the solvents alone. In both series, rats were killed 24 hr after the haloalkane challenge. Liver injury was assessed biochemically (plasma ALT activities and bilirubin concentrations) and morphologically. When TCE was added to a solution of CCl_4, smaller doses of CCl_4 were required to produce equally severe liver injury. The MED of acetone required to potentiate CCl_4-induced liver injury was at least five times smaller when TCE (0.25 ml/kg) was added to the challenge solution of CCl_4. The severity of the injury produced by TCE–CCl_4 mixtures administered in different proportions was constant, and potentiated to the same extent by a given dose of acetone. These observations suggest that the presence of haloalkane mixtures can result in severe liver injury with lower doses of hepatotoxicants. They further illustrate that prior exposure to acetone may markedly affect the response elicited by the haloalkane mixture.

Haloalkanes are extensively present in the industrial environment, and several of them are known to produce liver injury in humans and in animals. Ketonic (e.g., acetone) or ketogenic compounds can potentiate haloalkane-induced hepatotoxicity (Hewitt *et al.,* 1980; Pilon *et al.,* 1986); trichloroethylene (TCE) and carbon tetrachloride (CCl_4) figure among the haloalkanes whose toxicity is potentiated. The minimal effective dosage (MED) of acetone required to potentiate a challenge of CCl_4 (0.1 ml/kg) in rats was previously reported to be 0.25 ml/kg (Plaa *et al.,* 1982; Charbonneau *et al.* 1986a). Pessayre *et al.* (1982) reported that rats treated intraperitoneally with a mixture of nontoxic dosages of

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TCE and CCl_4 exhibited a moderate to severe liver injury. Their findings were consistent with the contention that the liver damage observed after the administration of TCE–CCl_4 mixtures resulted from a TCE enhancement of CCl_4 hepatotoxicity. They also showed that when the TCE or CCl_4 dose is kept constant, the severity of the liver injury is dependent on the dose of the other haloalkane in the mixture. More recently, Charbonneau *et al.* (1986b) demonstrated that acetone strongly potentiates the hepatotoxicity induced by a mixture of TCE and CCl_4.

The aim of the present study was to define more precisely the interaction between acetone, TCE, and CCl_4. Two objectives were pursued: (a) To determine if the MED of acetone required to potentiate CCl_4 liver injury is smaller when the challenge is a mixture of TCE and CCl_4 instead of CCl_4 alone. (b) To determine if the severity of the acetone-potentiated TCE–CCl_4-induced liver injury was altered when the dosage of CCl_4 is decreased but accompanied by a concomitant increase of the TCE dosage in the mixture. Liver injury was assessed by biochemical indices and a semiquantitative morphological analysis.

METHODS

Animal treatments. Male Sprague–Dawley rats (175–200 g) were purchased from Charles River Canada, Inc. (St. Constant, Québec), and maintained on Charles River rat chow No. 5012 and water *ad libitum*. Animals were used after a 4-day acclimation period in animal quarters maintained on a 12-hr light/dark cycle. The rats were housed (six per cage) in stainless steel open-bottom cages. The experimental unit consisted of six rats. During the treatment period, animals received a single po injection (3:00 PM) of corn oil (10 ml/kg) or acetone solution; the solutions were prepared such that 10 ml of solution/kg was administered. In a first series of experiments (variation of the MED), the acetone dosages employed were 0.05, 0.10, 0.15, 0.20, and 0.25 ml/kg (0.7, 1.4, 2.0, 2.7, and 3.4 mmol/kg, respectively). Rats in the second series of experiments were dosed with acetone (0.75 ml/kg) (10.2 mmol/kg). After an 18-hr treatment period, the rats were challenged with an ip injection of haloalkane in both experiments. Plaa and Hewitt (1982) reported that acetone potentiation of $CHCl_3$-induced liver injury was maximal when the challenge was administered 18 hr later. Traiger and Plaa (1971) showed that the potentiation of CCl_4-induced liver injury by isopropanol, a ketogenic substance producing acetone, was maximal when the challenge was administered 18–24 hr later. The CCl_4 solutions in corn oil were prepared such that the rats received 4 ml of solution/kg. Rats in the first series were challenged with either (a) corn oil (4 ml/kg), (b) TCE (0.25 ml/kg) (2.8 mmol/kg), (c) CCl_4 (0.1 ml/kg) (1.0 mmol/kg), (d) TCE (0.25 ml/kg)–CCl_4 (0.1 ml/kg). Rats in the second series were challenged with TCE–CCl_4 mixtures prepared by using different percentages (25, 50, or 75%) of a weakly toxic dosage determined for each haloalkane; the 100% dosages selected were 1.5 ml/kg (16.7 mmol/kg) for TCE and 0.25 ml/kg (2.6 mmol/kg) for CCl_4. The different mixtures were (A) 25% TCE–75% CCl_4, (B) 50% TCE–50% CCl_4, (C) 75% TCE–25% CCl_4. Animals were weighed 24 hr later and lightly anesthetized with ether, and blood was removed via the abdominal aorta with heparin as the anticoagulant.

Biochemical analyses. Alanine aminotransferase (ALT) activity was determined in aliquots of plasma by the method of Reitman and Frankel (1957), using a Dade Chemical Co. reagent kit. Plasma total bilirubin concentrations were determined by the Jendrassik method (Jendrassik and Grof, 1938; Nosslin, 1960), using an American Monitor kit.

Morphological analyses. After fixation in 10% buffered Formalin, sections of the lateral median lobe of the liver were dehydrated and embedded in paraffin. Coronal sections (5 μm) from the paraffin-embedded tissue were cut and stained with hematoxylin–eosin. Quantitative analysis was performed by observing 10 fields for each slide (one slide per rat; six rats per group) at 100× magnification, and measuring the distance occupied by ballooning, necrotic, and normal hepatocytes between the central vein and the portal triad as previously described by Iijima *et al.* (1983).

Statistical analysis. A log (X) transformation was applied to ALT values prior to statistical analysis to normalize response variables and reduce treatment variances, as evaluated by Levene's test for homogeneity of variance. Biochemical data used to determine the MED were submitted to a 6 × 4 (potentiator, challenge) factorial analysis of variance followed by a Newman–Keuls multiple range test; $\alpha = 0.05$ was used as the level of significance.

RESULTS

Variation of Acetone MED

Table 1 depicts the results obtained for corn oil—or acetone (0.05–0.25 ml/kg)—pretreated rats challenged with corn oil, TCE, CCl_4, or a TCE–CCl_4 mixture. No significant increases in plasma ALT activities and bilirubin concentrations were observed in the

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TABLE 1

.FFECT OF ACETONE DOSAGE ON PLASMA ALT ACTIVITY AND BILIRUBIN CONCENTRATION MEASURED 24 hr AFTER A CHALLENGE OF CORN OIL, TCE, CCl_4, OR A MIXTURE OF TCE–CCl_4

:eatment ml/kg)	Challenge			
	Corn oil	TCE	CCl_4	TCE–CCl_4
ı oil	36 ± 4[a] 0.29 ± 0.01[b]	45 ± 4 0.29 ± 0.02	51 ± 6 0.29 ± 0.03	144 ± 17 0.25 ± 0.03
·one (0.05)	40 ± 1 0.25 ± 0.01	31 ± 2 0.26 ± 0.02	81 ± 11 0.15 ± 0.01	395 ± 90[c] 0.38 ± 0.02
:one (0.10)	39 ± 2 0.27 ± 0.04	45 ± 2 0.30 ± 0.04	79 ± 9 0.32 ± 0.03	523 ± 70[c] 0.21 ± 0.02
tone (0.15)	38 ± 5 0.25 ± 0.01	42 ± 9 0.25 ± 0.02	94 ± 13 0.31 ± 0.02	699 ± 200[c] 0.45 ± 0.04[c]
tone (0.20)	34 ± 1 0.25 ± 0.02	36 ± 1 0.22 ± 0.02	65 ± 10 0.36 ± 0.02	736 ± 276[c] 0.49 ± 0.06[c]
:tone (0.25)	36 ± 3 0.28 ± 0.02	40 ± 3 0.25 ± 0.02	172 ± 35[c] 0.16 ± 0.02	706 ± 76[c] 0.48 ± 0.05[c]

'ote. Rats were treated orally with corn oil (10 ml/kg) or acetone and were challenged (ip) 18 hr later with corn oil ml/kg), trichloroethylene (TCE, 0.25 ml/kg), CCl_4 (0.1 ml/kg), or a [TCE (0.25 ml/kg)–CCl_4 (0.1 ml/kg)] mixture. ısma samples were obtained 24 hr after the challenge.

[a] Upper row values represent mean ± SE of plasma ALT activity U/ml (six animals/group).

[b] Lower row values represent mean ± SE of plasma bilirubin concentration mg/dl (six animals/group).

[c] Significantly larger ($p < 0.05$, Newman–Keuls multiple range test) than the corn oil-pretreated group receiving .ı identical challenge.

groups challenged with corn oil or TCE 0.25 ml/kg. Corn oil-pretreated rats challenged with a TCE–CCl_4 mixture yielded a mean plasma ALT activity three-fold higher ($p < 0.05$) than CCl_4-challenged rats, whereas plasma bilirubin concentrations were not significantly different ($p > 0.05$) between these two groups. These results are in accord with those previously reported by Pessayre *et al.* (1982) and Charbonneau *et al.* (1986b).

The acetone MED required to potentiate the haloalkane-induced liver injury was determined as the smallest dose for which the statistical analysis revealed a significantly increased ($p < 0.05$) plasma ALT activity in comparison to the corn oil-pretreated group receiving an identical haloalkane challenge. The MED of acetone for a challenge of CCl_4 (0.1 ml/kg) alone was equal to 0.25 ml/kg (Table 1). This agrees with the findings previously reported by this laboratory (Plaa *et al.*, 1982; Charbonneau *et al.* 1986a). When the haloalkane challenge was a TCE–CCl_4 mixture, the MED of acetone, however, was equal to or lower than 0.05 ml/kg (Table 1). Variations in the response of the TCE–CCl_4 group prompted us not to test smaller acetone dosages. Therefore, the addition of TCE (0.25 ml/kg) to the CCl_4 solution resulted in at least a five-fold decrease of the acetone MED required for potentiation of CCl_4 liver injury. Furthermore, the resulting plasma ALT activities and bilirubin concentrations of the [acetone (0.05 ml/kg) + TCE–CCl_4]-treated group were significantly higher ($p < 0.05$) than the value obtained for the [acetone (0.25 ml/kg) + CCl_4]-treated group.

The morphological analysis was performed by measuring the distance occupied by necrotic, ballooning, and normal hepatocytes in the hepatic lobule; the ratio of necrotic to normal hepatocytes was calculated. This ratio is a useful parameter to quantify the major changes in treated rats, since both types of

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TABLE 2

HISTOLOGICAL EVALUATION OF THE EFFECTS OF ACETONE DOSAGE ON CCl_4 AND TCE–CCl_4 LIVER INJURY IN MALE RATS

Dosage of acetone (ml/kg)	CCl_4				TCE–CCl_4			
	Nec	Bal	Nor	Nec/Nor	Nec	Bal	Nor	Nec/Nor
0.00	0	173	256	0.00	80	194	205	0.39
0.05	26	83	217	0.12	119	178	159	0.75
0.10	35	138	316	0.11	131	175	165	0.79
0.15	24	148	239	0.10	114	181	162	0.70
0.20	29	183	209	0.14	99	151	174	0.57
0.25	41	173	200	0.21	132	130	157	0.84

Note. Rats were pretreated orally with acetone (0.05, 0.10, 0.15, 0.20, or 0.25 ml/kg) and were challenged (ip) 18 hr later with CCl_4 (0.1 ml/kg) or a [TCE (0.25 ml/kg)–CCl_4 (0.1 ml/kg)] mixture. Liver samples were obtained 24 hr after the challenge. Results are expressed in distance (μm) occupied by cell type from the portal triad to the central vein; values represent the mean of 10 observations/animal for six rats/group. Nec, necrotic hepatocytes; Bal, ballooning of hepatocytes; Nor, normal hepatocytes.

acute alterations (necrosis with inflammatory cell infiltration and ballooning cells including vacuolization) are evaluated in a unique measurement. For a constant number of necrotic cells, a larger number of balloon cells leads to a smaller number of normal cells; therefore the calculated ratio is larger.

The data in Table 2 show that the [necrosis/normal] ratio was nearly two-fold higher in CCl_4-challenged rats pretreated with acetone (0.25 ml/kg = MED) than in acetone (0.05–0.20 ml/kg)-pretreated animals, and represented a mild increase in comparison to corn oil-pretreated rats (no necrosis). Corn oil-pretreated rats challenged with a mixture of TCE–CCl_4 yielded a ratio higher than that of those challenged with only CCl_4. The acetone (0.05–0.25 ml/kg)-pretreated groups challenged with a mixture of TCE–CCl_4 yielded ratios that were 3.5–4.0 times larger than those of the rats challenged with only CCl_4 and receiving the MED dosage of acetone (0.25 ml/kg) (Table 2). Therefore, the morphological findings confirmed that the MED of acetone is decreased when TCE was added to the solution of CCl_4.

Mixture Composition on TCE-CCl_4-Induced Liver Injury

The addition of TCE to a CCl_4 solution (0.1 ml/kg), as discussed above, reduces the MED of acetone required for potentiation of the haloalkane mixture-induced liver injury. Thus, it appeared important to determine whether the CCl_4 dose could also be lowered when larger doses of TCE are used. To address this problem, three mixtures of different proportions of TCE and CCl_4 were prepared. CCl_4 (0.25 ml/kg) alone was first selected as the 100% dose. Plasma ALT activities measured for these rats was slightly increased above normal [Fig. 1 vs Table 1 (corn oil + corn oil)], indicating a mild hepatotoxic response. TCE (1.5 ml/kg) was used as the 100% TCE dose and was shown to be a very weak hepatotoxicant [Fig. 1 vs Table 1 (corn oil + corn oil)].

Mixtures were prepared using CCl_4 dosages equal to 75, 50, and 25% of the 0.25-ml/kg dosage; the decreases in the CCl_4 dosage were accompanied by a concomitant increase of the TCE dosage equal to 25, 50, and 75% of the 1.5 ml TCE/kg dosage, (Figs. 1–3 and Table 3). Plasma ALT activities measured in corn oil-pretreated rats challenged with (dosages in ml/kg) [TCE (0.38)–CCl_4 (0.19)], [TCE (0.75)–CCl_4 (0.13)], or [TCE (1.11)–CCl_4 (0.06)] were similar to each other, but approximately 13 times higher than the activity obtained for CCl_4 (0.25 ml/kg)-treated rats (Fig. 1). Histological analysis showed that the

CCl4

:-CCl4	
Nor	Nec/Nor
205	0.39
159	0.75
165	0.79
162	0.70
174	0.57
157	0.84

were challenged (ip) 18 ples were obtained 24 hr ortal triad to the central :patocytes: Bal, balloon-

for potentiation of ıduced liver injury. rtant to determine uld also be lowered E are used. To ad- nixtures of different :Cl4 were prepared. .vas first selected as .LT activities mea- slightly increased Table 1 (corn oil nild hepatotoxic re-) was used as the shown to be a very . 1 vs Table 1 (corn

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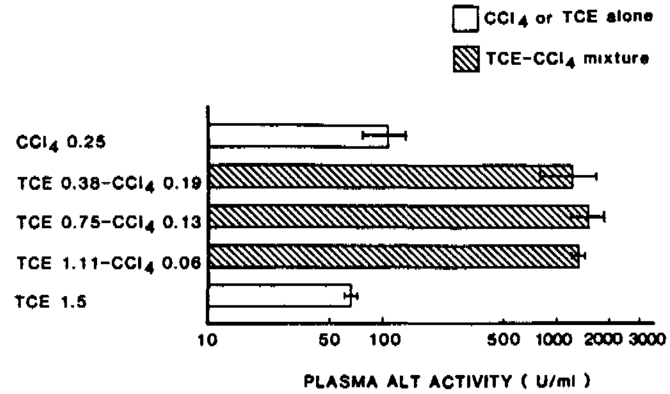

FIG. 1. Plasma ALT activities measured in corn oil-pretreated rats challenged with TCE–CCl_4 mixtures of different proportions. Rats were pretreated orally with corn oil (10 ml/kg) and were challenged (ip) 18 hr later with CCl_4 (0.25 ml/kg), TCE (1.5 ml/kg), or a TCE–CCl_4 mixture: (dosages in ml/kg) [TCE (0.38)–CCl_4 (0.19)], [TCE (0.75)–CCl_4 (0.13)], or [TCE (1.11)–CCl_4 (0.06)]. Plasma samples were obtained 24 hr after the challenge. Results represent the mean ± SE for six animals/group.

necrosis/normal) ratio was slightly smaller when the CCl_4 dosage in the mixture was decreased (Table 3); the (necrosis/normal) ratios for the TCE–CCl_4 mixtures were 1.9–2.4 times higher than the ratio measured in rats challenged with only CCl_4 (0.25 ml/kg). Administration of a high dose of TCE (0.11 ml/kg) in a mixture with a low dose of CCl_4 (0.06 ml/kg) yielded a mean plasma ALT activity and a (necrosis/normal) ratio that was markedly increased when compared to the values obtained with a small dose (0.1 ml/kg) of CCl_4 given alone (1355 ± 104 vs 51 ± 6 and 0.71 vs 0.00, respectively), showing the strong enhancing effect of TCE. These results demonstrate that low doses of the hepatotoxicant, CCl_4, can still induce severe liver injury when administered as a mixture with high doses of a very weak hepatotoxicant, TCE.

Acetone-pretreated rats challenged with CCl_4 (0.25 ml/kg) exhibited severe liver injury, whereas animals challenged with TCE

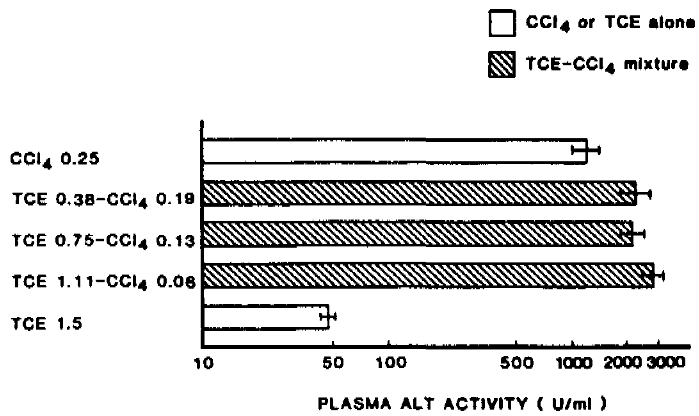

FIG. 2. Plasma ALT activities measured in acetone-pretreated rats challenged with TCE–CCl_4 mixtures of different proportions. Rats were pretreated orally with acetone (0.75 ml/kg) and were challenged (ip) 18 hr later with CCl_4, TCE, or a TCE–CCl_4 mixture (see Fig. 1 for dosages). Plasma samples were obtained 24 hr after the challenge. Results represent the mean ± SE for six animals/group.

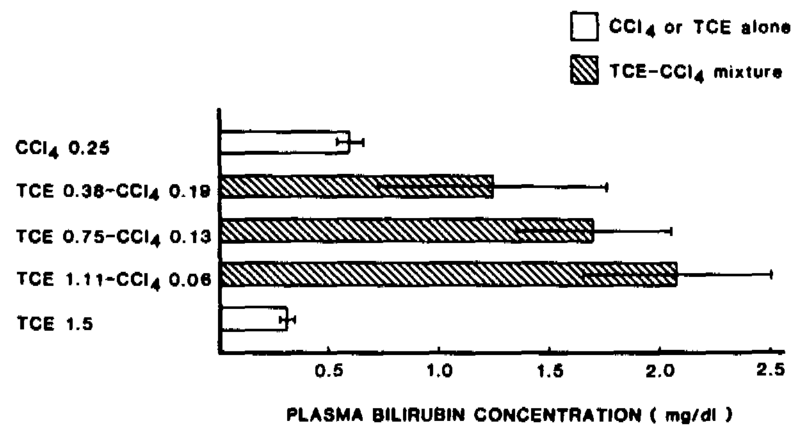

FIG. 3. Plasma bilirubin concentrations measured in acetone-pretreated rats challenged with TCE–CCl_4 mixtures of different proportions. Rats were pretreated orally with acetone (0.75 ml/kg) and were challenged (ip) 18 hr later with CCl_4, TCE, or a TCE–CCl_4 mixture (see Fig. 1 for dosages). Plasma samples were obtained 24 hr after the challenge. Results represent the mean ± SE for six animals/group.

(1.5 ml/kg) showed no hepatotoxicity (Fig. 2 and Table 3). Plasma ALT activities measured in acetone-pretreated rats challenged with (doses in ml/kg) [TCE (0.38)–CCl_4 (0.19)], [TCE (0.75)–CCl_4 (0.13)], or [TCE (1.11)–CCl_4 (0.06)] were similar to each other, and were increased twofold compared to CCl_4 (0.25 ml/kg)-treated rats (Fig. 2). Histological evaluation of liver injury in acetone-pretreated rats revealed a more severe response in rats challenged with only CCl_4 (0.25 ml/kg) than those treated with any of the three mixtures studied (Table 3). The results suggest that none of the three mixtures studied was subjected to a greater potentiating effect of the haloalkane-induced liver injury, although the severe response obtained with CCl_4 (0.25 ml/kg) alone (45% of the lobule being composed of necrotic hepatocytes) restricted the possibility of observing further marked increases.

The cholestatic component of the liver lesion, indirectly evaluated by the increase of plasma total bilirubin concentration, was un-

TABLE 3

HISTOLOGICAL EVALUATION OF THE EFFECTS OF TCE AND CCl_4 DOSAGE ON THE SEVERITY OF HALOALKANE MIXTURE-INDUCED LIVER INJURY IN MALE RATS PRETREATED WITH CORN OIL OR ACETONE

Dosage of TCE (ml/kg)	Dosage of CCl_4 (ml/kg)	Corn oil-pretreated rats				Acetone-pretreated rats			
		Nec	Bal	Nor	Nec/Nor	Nec	Bal	Nor	Nec/Nor
0	0.25	68	179	182	0.37	196	103	135	1.45
0.38	0.19	162	70	184	0.88	200	29	171	1.17
0.75	0.13	120	130	162	0.74	193	0	207	0.93
1.11	0.06	126	108	178	0.71	238	0	184	1.29
1.5	0	0	0	396	0	0	0	432	0

Note. Rats were pretreated orally with corn oil (10 ml/kg) or acetone (0.75 ml/kg) and were challenged 18 hr later with CCl_4, TCE, or a TCE–CCl_4 mixture. Liver samples were obtained 24 hr after the challenge. Results are expressed in distance (μm) occupied by cell type from the portal triad to the central vein; values represent the mean of 10 observations/animal for six rats/group. Nec, necrotic hepatocytes; Bal, ballooning of hepatocytes; Nor, normal hepatocytes.

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ITY OF HALOALKANE
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Nor	Nec/Nor
135	1.45
171	1.17
207	0.93
184	1.29
432	0

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DISCUSSION

It was previously reported that CCl_4-in- uced hepatotoxicity is enhanced by the si- nultaneous administration of TCE (Pessayre t *al.*, 1982), and that this response is potenti- ted by pretreatment with acetone (Charbon- neau *et al.*, 1986b). In the present study, ex- periments were performed to verify if the MED of acetone required to potentiate CCl_4-induced liver injury is decreased when the haloalkane challenge is a mixture of TCE and CCl_4 instead of CCl_4 alone. The results show that the MED of acetone was decreased from 0.25 to 0.05 ml/kg, or lower, when TCE (0.25 ml/kg) was added to the CCl_4 (0.1 ml/kg) solution (Tables 1 and 2). Thus, smaller amounts of the ketone are required to potentiate the TCE–CCl_4 hepatotoxic response than those needed to potentiate CCl_4 toxicity.

The relevance of this observation to the occupational setting may be important. We previously reported (Charbonneau *et al.*, 1986a) that in rats the severity of ketone-potentiated CCl_4 liver injury resulting from acetone exposure by inhalation (2500 ppm for 4 hr) is equal to that obtained after a single oral administration of acetone of 0.25 ml/kg (MED); the injury arising from larger inhalation exposures (multiples of the MED) correlate very well with the corresponding oral administration dosages of acetone. Since the MED of acetone was shown to be five times lower when TCE was added to the CCl_4 solution in the present study, one can infer that the corresponding acetone MED for exposures by inhalation would be 500 ppm (4 hr exposure). Recently, Landry and Brodeur (unpublished data) observed that exposure of rats to acetone by inhalation (500 ppm for 4 hr) resulted in potentiated liver injury, when they were challenged with CCl_4 (2500 ppm for 6 hr) 18 hr later. Extrapolation of these data to humans in the occupational setting suggests that such an ambient concentration of acetone would be below the recommended threshold limit value of 750 ppm (8 hr/day, 5 days/week) for industrial exposure. Thus, the acetone dose involved in the potentiation phenomenon falls within that which might arise from exposure in the workplace.

Small doses of acetone and TCE, two widely used solvents, simultaneously administered with CCl_4 can lead to liver injury. It is probably of greater importance, however, to verify if the administration of larger doses of these solvents combined with the administration of a smaller dose of the hepatotoxicant results in liver injury. The data show that, compared to rats challenged with CCl_4 alone, a smaller dose of CCl_4 produced severe liver injury when administered in a mixture with proportionately increasing doses of TCE (Fig. 1). In all cases, pretreatment with the larger dose of acetone prior to the challenge led to a more severe liver injury (Fig. 2).

Normally, CCl_4 liver injury, resulting in extensive necrosis, is not associated with marked increases in plasma bilirubin retention (Traiger and Plaa, 1971). However, a small dose of CCl_4 potentiated by isopropanol, which is biotransformed to acetone (Traiger and Plaa, 1982), produces markedly elevated plasma bilirubin concentrations (Traiger and Plaa, 1971); de Lamirande and Plaa (1981) demonstrated that isopropanol enhances the cholestatic as well as the necrogenic properties of CCl_4. Thus, with CCl_4, elevated plasma bilirubin concentrations are an indirect indication of the cholestatic properties of this haloalkane. Interestingly, results shown in Fig. 3 indicate that elevated plasma bilirubin levels were observed when the potentiation was more severe: [TCE (1.11)–CCl_4 (0.06)] > [TCE (0.75)–CCl_4 (0.13)] > [TCE (0.38)–CCl_4 (0.19)]. These observations suggest that the liver injury resulting from the potentiation are associated with a marked cholestatic component as well as extensive necrosis.

Exposures to haloalkane mixtures may be frequent in the occupational setting. Furthermore, acetone is quite widely used industrially. The interactive phenomenon described in the present study is quite evident, but its application to the workplace is yet to be established. It is already evident, however, that the rule of adjusting TLVs for mixtures of chemicals on the assumption that their effects are additive (ACGIH, 1986) is not valid for all combinations of such chemicals. Further research is needed to determine if the observations reported herein can be observed with other haloalkanes. Finally, if they can be observed in laboratory animals, one needs to establish the likelihood that the interactions might occur with chemical exposure patterns that exist in occupational environments.

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