Potentiation of Non-Halocarbon Oxidants on Halocarbon-Induced Oxidative Damage to Rat Red Blood Cells

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We have studied individual and mixture toxicities of halocarbons and non-halocarbons in rat red blood cells (rRBC). Hemolysis and release of TBARS, hemoglobin (Hb), and methemoglobin (MetHb) as well as release of glutathione peroxidase and catalase were determined. Tetrahalocarbons were more damaging than dihalocarbons, and bromoethanes were more damaging to rRBC than chloroethanes. *tert*-Butyl hydroperoxide (BHP) was most damaging to rRBC by every measurement, while H_2O_2 had no effect. 2,2-Azobis(2-amidinopropane) hydrochloride (AAPH) increased MetHb in the medium but decreased hemolysis. BHP strongly potentiated the damage of rRBC induced by $BrCCl_3$ (0.5 mM), including a 100% hemolysis within 2 h at 37 °C. BHP potentiated the release of MetHb from rRBC incubated with $C_2H_2Br_4$ or $C_2H_2Cl_4$ but not with $C_2H_4Br_2$ or $C_2H_4Cl_2$. The potentiation of BHP on the release of MetHb was selective as BHP did not affect lipid peroxidation or hemolysis. AAPH significantly inhibited hemolysis induced by $C_2H_2Br_4$ and $C_2H_2Cl_4$ and release of Hb induced by $C_2H_2Cl_4$. AAPH did not affect lipid peroxidation or MetHb release induced by these halocarbons. Thus, the mixture of a halocarbon and a non-halocarbon led to mostly potentiating toxicity, although antagonistic effects could occur depending on the type of oxidant mixture used. Moreover, lipid peroxidation and Hb oxidation were selectively affected by the oxidant mixture.

Halogenated hydrocarbons have been widely used as solvents for decades, and they are found in drinking water, air, and foods as a result of such use (Reynolds et al., 1980; *Chemical & Engineering News*, 1984). Many halocarbons are known inducers of lipid peroxidation both in vitro and in vivo (Reynolds et al., 1980). Among them CCl_4 has been studied extensively and has been shown to be metabolized by the microsomal mixed function oxidases (Reynolds et al., 1980; Recknagel et al., 1977).

Most studies dealing with toxicity of halocarbons have employed a single compound rather than a mixture with another halocarbon or a non-halocarbon oxidant. In reality, however, humans are likely to be exposed to more than one toxicant in the environment and workplace. The importance of studying mixture toxicities has been recognized (Bingham and Morris, 1988; Sano and Tappel, 1990). Several chlorinated hydrocarbons have been reported to potentiate CCl_4-induced hepatotoxicity (Plaa, 1980; Harris et al., 1982; Kefalas and Stacey, 1989, 1991) and toxicity in plasma membranes (Kefalas and Stacey, 1991). In addition, Sano and Tappel (1990) have shown that some halocarbons synergistically enhanced lipid peroxidation in rat liver slices induced by *tert*-butyl hydroperoxide (BHP).

Hidalgo et al. (1990) have recently examined 25 halocarbons for lipid peroxidation and hemoglobin (Hb) oxidation in isolated rat red blood cells (rRBC). They show that rRBC is a simple and useful biological system for studying halocarbon toxicity. In the present studies, we used rRBC to investigate mixture toxicities by combining a halocarbon with a non-halocarbon oxidant including 2,2-azobis(2-amidinopropane) hydrochloride (AAPH), H_2O_2, and BHP. BHP, an amphiphatic molecule, effectively causes lipid peroxidation and hemoglobin degradation in red cells (Trotta et al., 1982, 1983; Suzuki et al., 1988). AAPH, a water-soluble peroxy radical generator (Niki, 1990), induces lipid peroxidation, protein oxidation, and hemolysis in erythrocytes (Yamamoto et al., 1986; Miki et al., 1987). H_2O_2 is a water-soluble physiological oxidant and a component of the Fenton reaction system which generates hydroxyl radicals. We hypothesized that combination of a hydrophobic halocarbon with an aqueous non-halocarbon oxidant would potentiate oxidative damage to rRBC.

MATERIALS AND METHODS

$BrCCl_3$ was obtained from Eastman Kodak Co. (Rochester, NY), and $C_2H_2Cl_4$ was from J. T. Baker Chem. Co. (Phillipsburg, NJ). $C_2H_4Br_2$, $C_2H_2Br_4$, and $C_2H_4Cl_2$ were purchased from Aldrich Chemical Co. (Milwaukee, WI), and BHP and AAPH were from Polysciences Inc. (Warrington, PA).

Male Sprague-Dawley rats weighing 250–300 g (Bantin and Kingman, Fremont, CA) were anesthetized with ether, and blood was obtained from the abdominal aorta through a heparinized needle and mixed with 3% sodium citrate at 0.1 volume of blood. Red blood cells were isolated by centrifugation at 3000*g* for 5 min at 4 °C and washed twice with 0.9% NaCl. The packed cells were then suspended in phosphate-buffered saline (PBS), pH 7.4 (Draper and Csallany, 1969), containing 5 mM glucose and saturated with oxygen.

Suspended rRBC were transferred to a test tube with a Teflon cap and mixed gently with small aliquots of oxidants to a total volume of 5.0 mL. The final concentration of rRBC was 2% (v/v). Oxidants used included BHP (dissolved in PBS), AAPH (dissolved in PBS), and halogenated hydrocarbons [dissolved in dimethyl sulfoxide (DMSO)]. The final concentration of DMSO used (0.1%) did not affect the biochemical assays. The rRBC were preincubated at 37 °C for 5 min before the addition of oxidants to start the reaction. Incubations were carried out for 2 h, unless otherwise indicated, at 37 °C in a water bath with shaking (160 cycles/min).

Following incubation the cells were discarded by centrifugation at 3000*g* for 5 min at 4 °C. Supernatants were carefully aspirated for measurements of hemolysis, Hb oxidation, lipid peroxidation, and release of enzymes. Hemolysis was determined as described by Draper and Csallany (1969). Total hemoglobin (Hb) and methemoglobin (MetHb) were determined using the method of Evelyn and Malloy (1938) with some modifications (Henry, 1964). Lipid peroxidation was determined as thiobarbituric acid-reactive substances (TBARS) using a fluorometric method of Yagi (1976) with slight modification as described before (Sano et al., 1986).

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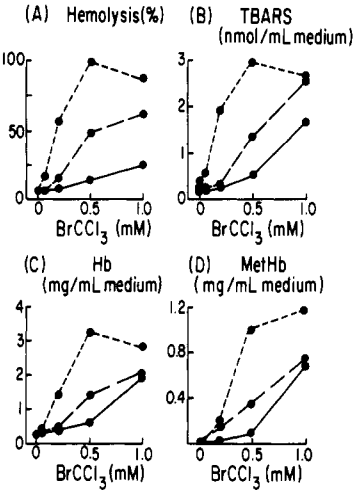

Figure 1. Hemolysis of and release of TBARS. Hb and MetHb from rRBC incubated with $BrCCl_3$ (0, 0.05, 0.20, 0.50 and 1.0 mM) alone (—) and in the presence of 0.05 (– –) or 0.10 mM BHP (- - -). Each point is the mean of four to six replications (standard deviation ≤10%).

Glutathione peroxidase (GPx) (Lawrence and Burk, 1976) and catalase (Worthington, 1988) were determined using published methods.

Data were pooled from different rats for each replicate. Statistical analysis was carried out using a computerized program (SAS Institute, Cary, NC). Two-way analysis of variance (ANOVA) with a general linear model was used to determine the main effects and interactions of oxidants. Barlett's test was used prior to the two-way ANOVA to ensure the homogeneity of the data. Dunnett's test was also used when individual means were compared with the control group (no addition of oxidants) (Tables V and VI). The criterion of significance was $P < 0.05$ for all comparisons.

When two oxidants were used, percent interaction was calculated from the mean values as described previously (Sano and Tappel, 1990). Briefly, the formula was expressed as follows using an example of $BrCCl_3$ and BHP:

$$100\% \times \{[(BrCCl_3 + BHP) - none]/[(BrCCl_3 - none) + (BHP - none)] - 1\}$$

By this calculation, a positive value represented potentiation and a negative value antagonism. Potentiation and antagonism were only calculated when there was a significant interaction between the two oxidants by two-way ANOVA.

RESULTS

Effect of $BrCCl_3$ and in Combination with BHP. Figure 1 shows that rRBC incubated with $BrCCl_3$ resulted in concentration-dependent increase in hemolysis (Figure 1A) and release of TBARS (Figure 1B), Hb (Figure 1C), and MetHB (Figure 1D) to the medium. These responses were enhanced by the addition of a small amount of BHP (0.05 mM) to $BrCCl_3$ and greatly enhanced by the addition of 0.1 mM BHP. Potentiation of BHP on $BrCCl_3$-induced rRBC damage was exemplified by the release of TBARS and Hb (Figure 2). Potentiation on TBARS was most evident when low concentrations of $BrCCl_3$ were combined with 0.05 mM BHP. This effect decreased with increasing $BrCCl_3$ concentrations. With 0.05 mM BHP, the highest potentiation (140%) was at 0.5 mM $BrCCl_3$, and the potentiation decreased at either low or high concentrations of $BrCCl_3$. For release of Hb, the highest potentiation was at 0.5 mM $BrCCl_3$ for both 0.05 and 0.10 mM BHP.

Release of GPx and catalase also followed the same patterns as those of TBARS and Hb when the enzyme activity was calculated on the basis of milliliters of incubation medium (Figure 3). The specific activity of

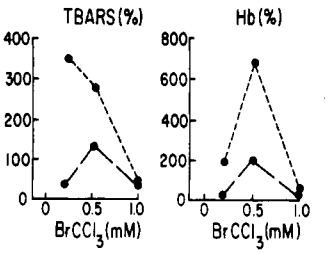

Figure 2. Percent potentiation of release of TBARS and Hb from rRBC incubated with $BrCCl_3$ (0.20, 0.50, and 1.0 mM) plus 0.05 (– –) and 0.10 mM BHP (- - -). Data are calculated from group means as described in the text.

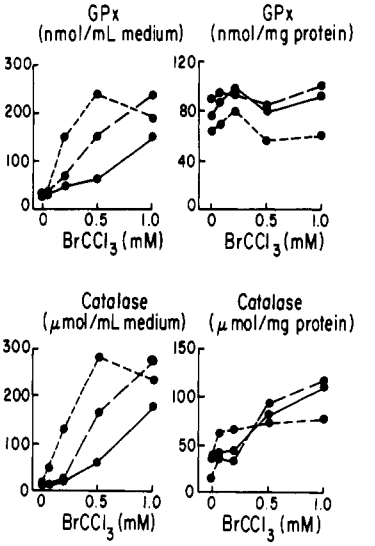

Figure 3. Release of enzymes from rRBC incubated with $BrCCl_3$ (0.05, 0.20, 0.50, and 1.0 mM) alone (—) or in the presence of 0.05 (– –) and 0.10 mM BHP (- - -). Each point is the mean of four to six replications (standard deviation ≤15%).

GPx (nanomoles of NADPH oxidized per milligram of protein in the medium) was little affected by increasing $BrCCl_3$ concentrations. Inclusion of 0.05 mM BHP resulted in no changes in the specific activity at each $BrCCl_3$ concentration. However, decreased activities occurred when $BrCCl_3$ was combined with 0.10 mM BHP. The specific activity of catalase was clearly increased with increasing concentration of $BrCCl_3$. The increase was not affected by the presence of 0.05 mM BHP but was largely negated by the presence of 0.10 mM BHP.

Interactions of $BrCCl_3$ with a Non-Halocarbon Oxidant. Interactions on hemolysis of and release of TBARS, Hb, and MetHb from rRBC were determined by combining $BrCCl_3$ (0.5 mM) with BHP (0.05 or 0.1 mM) or AAPH (2 or 10 mM). $BrCCl_3$ and BHP (at both 0.05 and 0.1 mM), but not H_2O_2 or AAPH, had a significant effect on hemolysis (Table I). There were significant interactions between BHP (at both 0.05 and 0.1 mM) and $BrCCl_3$ and between $BrCCl_3$ and 10 mM AAPH. Potentiation was highest for 1.0 mM BHP (579%), followed by 10 mM AAPH (198%) and 0.05 mM BHP (107%). H_2O_2 (1.0 mM) did not have a significant effect either alone or in combination with $BrCCl_3$.

In the absence of $BrCCl_3$, only 1.0 mM BHP caused a significant release of TBARS. AAPH and H_2O_2 were without effect (Table II). Both AAPH and BHP at the two concentrations tested had significant interactions with $BrCCl_3$, and the potentiation was 301% for 0.10 mM BHP, 140% for 10 mM AAPH, 113% for 2 mM AAPH, and 102% for 0.05 mM BHP.

The release of Hb and MetHb (Tables III and IV) induced by $BrCCl_3$ and a non-halocarbon oxidant was

Table I. Individual and Combined Effects of Bromotrichloromethane and a Non-Halocarbon on Hemolysis of rRBC

			AAPH		BHP	
	none	1 mM H_2O_2	2 mM	10 mM	0.05 mM	0.1 mM
control	9.77 ± 1.58[a]	8.43 ± 1.26	6.95 ± 1.13	4.90 ± 0.55	9.56 ± 1.18	10.48 ± 1.52
$BrCCl_3$ (BTM 0.5 mM)	23.15 ± 8.13	22.73 ± 5.55	27.11 ± 5.48	35.19 ± 5.48	37.07 ± 12.86	105.49 ± 1.93
interaction,[b] %				198	107	579
significant effects[c]						
H_2O_2		NS				
AAPH			NS	NS		
BHP					NS	NS
BTM		S	S	S	S	S
H_2O_2 × BTM		NS				
AAPH × BTM			NS	S		
BHP × BTM					S	S

[a] Values are means ± SD of four to six replications except for the control (n = 16). [b] Percent interaction was calculated as described in the text. [c] Two-way ANOVA: NS, not significant; S, significant.

Table II. Individual and Combined Effects of Bromotrichloromethane and a Non-Halocarbon on Medium TBARS Released from rRBC

			AAPH		BHP	
	none	1 mM H_2O_2	2 mM	10 mM	0.05 mM	0.1 mM
control	0.15 ± 0.06[a]	0.19 ± 0.04	0.20 ± 0.04	0.20 ± 0.07	0.21 ± 0.02	0.41 ± 0.14
$BrCCl_3$ (BTM 0.5 mM)	0.57 ± 0.16	0.65 ± 0.29	1.15 ± 0.26	1.28 ± 0.56	1.28 ± 0.56	2.88 ± 0.37
interaction,[b] %			113	140	102	301
significant effects[c]						
H_2O_2		NS				
AAPH			NS	NS		
BHP					NS	S
BTM		S	S	S	S	S
H_2O_2 × BTM		NS				
AAPH × BTM			S	S		
BHP × BTM					S	S

[a] Values are means ± SD of four to six replications except for the control (n = 12). [b] Percent interaction was calculated as described in the text. [c] Two-way ANOVA: NS, not significant; S, significant.

Table III. Individual and Combined Effects of Bromotrichloromethane and a Non-Halocarbon on Medium HB Released from rRBC

			AAPH		BHP	
	none	1 mM H_2O_2	2 mM	10 mM	0.05 mM	0.1 mM
control	0.23 ± 0.03[a]	0.23 ± 0.03	0.24 ± 0.09	0.17 ± 0.03	0.23 ± 0.03	0.24 ± 0.06
$BrCCl_3$ (BTM 0.5 mM)	0.58 ± 0.17	0.57 ± 0.16	0.85 ± 0.20	1.13 ± 0.14	1.03 ± 0.38	3.24 ± 0.10
interaction,[b] %			72	210	129	736
significant effects[c]						
H_2O_2		NS				
AAPH			NS	NS		
BHP					NS	NS
BTM		S	S	S	S	S
H_2O_2 × BTM		NS				
AAPH × BTM			S	S		
BHP × BTM					S	S

[a] Values are means ± SD of four to six replications except for the control (n = 12). [b] Percent interaction was calculated as described in the text. [c] Two-way ANOVA: NS, not significant; S, significant.

similar to that of TBARS. For Hb, potentiation was highest for 1.0 mM BHP (736%), followed by 10 mM AAPH (210%), 0.05 mM BHP (129%), and 2 mM AAPH (72%). For MetHb, potentiation was highest for 0.10 mM BHP (629%), followed by 0.05 mM BHP (149%), 10 mM AAPH (89%), and 2 mM AAPH (69%).

Interactions of Other Halocarbons with BHP and AAPH. When present alone at 1.0 mM, $C_2H_2Br_4$ was most effective in inducing hemolysis ($P < 0.05$) and release of TBARS ($P < 0.05$), Hb ($P < 0.05$), and MetHb ($P < 0.05$) (Tables V and VI). This was followed by $C_2H_2Cl_4$, which induced hemolysis ($P < 0.05$) and release of TBARS ($P < 0.05$) and Hb ($P < 0.05$) but not MetHb. Neither $C_2H_4Br_2$ nor $C_2H_4Cl_2$ produced any significant effects on these measurements. When added in combination with BHP (0.10 mM), none of the four halocarbons showed a significant interaction with BHP (0.10 mM) on hemolysis or lipid peroxidation. There, however, was a significant potentiation on MetHb between $C_2H_2Br_4$ and BHP (127%) and between $C_2H_2Cl_4$ and BHP (225%). A significant potentiation also occurred on the release of Hb between BHP and $C_2H_2Cl_4$ (34%) (Table VI).

AAPH at 10 mM, either alone or combined with a halocarbon (1.0 mM) other than $BrCCl_3$, did not affect lipid peroxidation but significantly lowered hemolysis (Table V). Antagonisms on hemolysis of 49 and 34% were found when AAPH was combined with $C_2H_2Cl_4$ and $C_2H_2Br_4$, respectively. A 22% antagonism on Hb release occurred when AAPH was combined with $C_2H_2Cl_4$ (Table VI). AAPH alone strongly induced release of MetHb, which was not affected by a combination with any of these four halocarbons.

DISCUSSION

Hidalgo et al. (1990) have recently shown that many halocarbons induce lipid peroxidation and Hb damage in

Table IV. Individual and Combined Effects of Bromotrichloromethane and a Non-Halocarbon on MetHb Released from rRBC

			AAPH		BHP	
	none	1 mM H_2O_2	2 mM	10 mM	0.05 mM	0.1 mM
control	0.011 ± 0.008[a]	0.027 ± 0.017	0.078 ± 0.016	0.097 ± 0.017	0.007 ± 0.003	0.021 ± 0.008
$BrCCl_3$ (BTM 0.5 mM)	0.137 ± 0.041	0.170 ± 0.066	0.325 ± 0.099	0.515 ± 0.124	0.278 ± 0.081	1.025 ± 0.095
interaction,[b] %			69	89	149	629
significant effects[c]						
H_2O_2		NS				
AAPH			S	S		
BHP					NS	NS
BTM		S	S	S	S	S
H_2O_2 × BTM		NS				
AAPH × BTM			S	S		
BHP × BTM					S	S

[a] Values are means ± SD of four to six replications except for the control (n = 12). [b] Percent interaction was calculated as described in the text. [c] Two-way ANOVA: NS, not significant; S, significant.

Table V. Individual and Combined Effects of Various Halogenated Hydrocarbons and a Non-Halocarbon on rRBC

	hemolysis, %			TBARS, nmol/mL of medium		
HHC	none	0.1 mM BHP	10 mM AAPH	none	0.1 mM BHP	10 mM AAPH
control	9.77 ± 1.58[a]	10.48 ± 1.52	4.90 ± 0.55[b]	0.15 ± 0.06	0.41 ± 0.14[b]	0.20 ± 0.07
$C_2H_4Br_2$ (1 mM)	12.08 ± 2.50	13.83 ± 1.28	6.02 ± 0.27	0.18 ± 0.09	0.40 ± 0.05	0.21 ± 0.01
$C_2H_2Br_4$ (1 mM)	51.63 ± 8.12[b]	46.82 ± 2.89	34.05 ± 16.25 (−34)	0.43 ± 0.13[b]	0.57 ± 0.05	0.43 ± 0.02
$C_2H_4Cl_2$ (1 mM)	10.27 ± 0.73	10.21 ± 0.47	8.20 ± 1.54	0.20 ± 0.02	0.38 ± 0.05	0.31 ± 0.01
$C_2H_2Cl_4$ (1 mM)	24.24 ± 5.87[b]	29.16 ± 6.67	14.66 ± 2.38 (−49)	0.32 ± 0.11[b]	0.54 ± 0.03	0.33 ± 0.03

[a] Values are means ± SD of four to six replications except for the control (n = 16). The values in parentheses are percent antagonism which shows a significant interaction between the two oxidants. [b] Significantly different from the control; $P < 0.05$.

Table VI. Individual and Combined Effects of Various Halogenated Hydrocarbons and a Non-Halocarbon on rRBC

	Hb, mg/mL of medium			MetHb, mg/mL of medium		
HHC	none	0.1 mM BHP	10 mM AAPH	none	0.1 mM BHP	10 mM AAPH
control	0.23 ± 0.03[a]	0.24 ± 0.06	0.17 ± 0.03	0.011 ± 0.008	0.021 ± 0.009	0.086 ± 0.020[b]
$C_2H_4Br_2$ (1 mM)	0.33 ± 0.05[b]	0.34 ± 0.07	0.33 ± 0.03	0.015 ± 0.005	0.033 ± 0.013	0.110 ± 0.020
$C_2H_2Br_4$ (1 mM)	1.15 ± 0.22[b]	1.13 ± 0.07	1.18 ± 0.08	0.030 ■ 0.006[b]	0.077 ± 0.021 (127)	0.105 ± 0.005
$C_2H_4Cl_2$ (1 mM)	0.27 ± 0.03	0.25 ± 0.03	0.35 ± 0.01 (500)	0.013 ± 0.004	0.025 ± 0.006	0.085 ± 0.007
$C_2H_2Cl_4$ (1 mM)	0.66 ± 0.05[b]	0.82 ± 0.08 (34)	0.52 ± 0.08 (−22)	0.017 ± 0.004	0.063 ± 0.028 (225)	0.100 ± 0.010

[a] Values are means ± SD of four to six replications except for the control (n = 12). The values in parentheses are percent interaction for data which have a significant interaction by two-way analysis of variance. [b] Significantly different from the control; $P < 0.05$.

rRBC. Their findings are interesting because halocarbons often require activation by the microsomal cytochrome P450 system (Reynolds et al., 1980; Recknagel et al., 1977) which is absent in rRBC. Possible mechanisms involved in activation of halocarbons in erythrocytes have been proposed (Hidalgo et al., 1990). In addition, release of TBARS from the rRBC is inversely correlated, while remaining oxyhemoglobin in rRBC is directly correlated, with the logarithm of LD_{50} of the halocarbons (Hidalgo et al., 1990). Thus, rRBC are useful to screen halocarbon toxicity. In the present studies, we have focused on oxidative damage to rRBC induced by a mixture of oxidants, i.e., a halocarbon and a peroxide or peroxyl radical generator.

Oxidative Damage Induced by Halocarbons. We first showed that brominated hydrocarbons were more damaging to rRBC than the chlorinated. This is consistent with findings from other in vitro systems (Knecht and Mason, 1991). It is tempting to explain the finding by relating to the difference in bond dissociation energy, that is, the C–Br bond dissociation energy is considerably smaller than that of the C–Cl bond (Slater and Sawyer, 1971; Koch et al., 1974). Thus, brominated hydrocarbons would undergo reductive dehalogenation and form reactive radical species more rapidly than would chlorinated analogues. However, it is not clear how halocarbons are activated in the rRBC.

Another interesting observation was that tetrahaloethanes caused more hemolysis and release of TBARS, Hb, and MetHb than the dihaloethanes. This is contrary to the findings in rat liver slices that dihaloethanes caused more release of TBARS than tetrahaloethanes (Sano and Tappel, 1990). The two different systems (rRBC vs rat liver slices) used may account for the discrepancy, since Hidalgo et al. (1990) also found higher MetHb in rRBC and more release of TBARS when rRBC were incubated with $C_2H_2Cl_4$ than with $C_2H_4Cl_2$. $C_2H_4Br_2$ does not generate oxyradicals or induce lipid peroxidation in vivo (Warren et al., 1991). We showed that neither $C_2H_4Br_2$ nor $C_2H_4Cl_2$ induced hemolysis, lipid peroxidation, or Hb oxidation in rRBC.

In hepatocytes incubated with CCl_4, GSH S-transferase was specifically released into the medium while GPx was not released (Lee et al., 1991). As both catalase and GPx, like TBARS and Hb moieties, were released into the incubation medium from rRBC, we suspect that the release of enzymes was nonspecific and was a result of hemolysis. The specific activity of catalase in the medium was activated by $BrCCl_3$ in a concentration-dependent manner. This is contrary to our expectation that catalase, a hemoprotein, would be susceptible, as would Hb, to oxidative damage induced by halocarbons.

Oxidative Damage Induced by Peroxides and AAPH. BHP is an effective organic peroxide that has often been used to study oxidative damage and antioxidant protection in erythrocytes (Trotta et al., 1982, 1983; Suzuki et al., 1988). BHP readily penetrates erythrocytes and forms butoxyl radicals by reacting with ferrous iron and

oxyhemoglobin in the cytosol (Suzuki et al., 1988). The radicals then attack the inner membrane, causing deterioration of membrane morphology, phospholipids, and cytoskeletal proteins, and eventually lead to hemolysis (Suzuki et al., 1988). Our results show that 0.1 mM BHP increased release of TBARS but did not affect hemolysis or release of Hb moieties. By contrast, H_2O_2, a physiological peroxide, at 1.0 mM had no effect on any of the measurements. The difference in damage to rRBC between H_2O_2 and BHP may be that the former has a low reaction rate constant with iron (to form hydroxyl radicals), while the latter is readily decomposed by reacting with iron or Hb to form noxious radicals (Gutteridge, 1987).

AAPH alone did not cause lipid peroxidation or release of Hb from rRBC, but it increased release of MetHb and decreased hemolysis. AAPH forms peroxyl radicals in the aqueous phase (Niki, 1990) and may thus react with cellular components that are released into the medium. As release of Hb was not increased, the increase in MetHb in the medium was likely a result of oxidation of Hb that was already present in the medium.

Interaction of Halocarbons with Non-Halocarbons. Sano and Tappel (1990) have shown that release of TBARS from liver slices induced by certain halocarbons was potentiated by BHP. In addition to lipid peroxidation, we have determined Hb oxidation and hemolysis of rRBC for the interaction of a halocarbon with a non-halocarbon oxidant. Moreover, the data of oxidant interactions were critically examined; i.e., the interaction (percent potentiation or antagonism) was calculated only when there was a statistically significant interaction between two oxidants.

BHP strongly potentiated lipid peroxidation and Hb oxidation of rRBC induced by $BrCCl_3$. Most markedly, 0.10 mM BHP plus 0.50 mM $BrCCl_3$ caused complete lysis of rRBC. The potentiation of $BrCCl_3$ by BHP (Figure 2) shows a lack of a dose-response relationship. This could be due to the high oxidation of Hb and lipid peroxidation induced by $BrCCl_3$ alone at high concentrations. Consequently, inclusion of another oxidant could not further potentiate the damage. $BrCCl_3$ also interacted with AAPH; the latter by itself did not cause lipid peroxidation or release of Hb from rRBC but increased release of MetHb and decreased hemolysis. When combined with $BrCCl_3$, AAPH potentiated not only lipid peroxidation and release of Hb and MetHb but also hemolysis. The in vivo significance of such an interaction is not clear since $BrCCl_3$ did not interact with H_2O_2 under the present experimental conditions.

BHP and AAPH also interacted with halocarbons in addition to $BrCCl_3$. BHP strongly potentiated the release of MetHb from rRBC incubated with $C_2H_2Br_4$ or $C_2H_2Cl_4$ but not with $C_2H_4Br_2$ or $C_2H_4Cl_2$. Such a potentiation of release of MetHb (and slightly so for Hb) was selective as BHP did not affect lipid peroxidation or hemolysis. The interaction of AAPH with halocarbons was more complex. AAPH significantly inhibited hemolysis and release of Hb induced by $C_2H_2Cl_4$. AAPH did not affect lipid peroxidation or MetHb release induced by these four halocarbons. Thus, the mixture interaction depended upon the type of oxidant mixture under investigation. In addition, lipid peroxidation and hemolysis were selectively affected, and the two events were not necessarily related. Our results do not allow interpretation of mechanism(s) by which halocarbons were metabolized and interacted with other oxidants. It could be that either the uptake or the activation of halocarbons in rRBC, by whatever mechanism(s), was affected (either enhanced or inhibited) by the presence of another oxidant.

In summary, we have shown that differential metabolism of halocarbons occurred in rRBC, namely, tetrahaloethanes were more damaging than dihaloethanes and bromoethanes were more damaging than chloroethanes. Combination of BHP with a halocarbon produced only potentiating effects, while combination of AAPH with a halocarbon produced both potentiating and antagonistic interactions. As mixture toxicities are highly complex and difficult to study in vivo, simple biological assays such as the present studies can be highly useful to show biological responses to a combination of toxicants.

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