SULPHYDRYL-DEPENDENT BIOTRANSFORMATION AND MACROMOLECULAR BINDING
OF 1,2-DIBROMO-2,4-DICYANOBUTANE IN BLOOD

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(Received March 27, 1998; accepted June 4, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

1,2-Dibromo-2,4-dicyanobutane (BCB) is a broad-spectrum microbicidal agent used commercially in consumer products. The objectives of this study were to elucidate the biotransformation of BCB, characterize its ability to covalently bind macromolecules, and predict the possible toxicological ramifications of such events. After iv administration of [14C]BCB to male Fischer 344 rats, 14C-equivalents were observed to bind gradually to blood constituents. The debrominated metabolite 2-methyleneglutaronitrile (MGN) was observed. In vitro experiments revealed that BCB was extremely labile and was readily debrominated in fresh whole blood, erythrocyte preparations, and buffered glutathione (GSH) solutions. In each case, the formation of MGN was inhibited by the alkylation of free sulfhydryls with N-ethylmaleimide (NEM). For every 1 mol of BCB converted to MGN, 2 mol of GSH were oxidized to glutathione disulfide (GSSG) (BCB + 2 GSH → MGN + GSSG + 2 HBr). The oxidation of free sulfhydryls during the conversion of BCB to MGN caused erythrocyte hemolysis (EC50 ~ 1 mM) in isolated preparations. Hemolysis was increased by coincubation of BCB with NEM (EC50 ~ 0.3 mM) and was decreased by coincubation with GSH (EC50 > 3 mM). However, MGN did not cause hemolysis of erythrocytes, even at concentrations 10-fold higher than the EC50 of BCB. In vitro experiments also demonstrated that incubation with either BCB or MGN resulted in significant macromolecular binding to the erythrocyte fraction of the blood (~80%). Incubation with NEM resulted in a significant decrease in binding for both BCB (11.3% bound) and MGN (29.5% bound). Because BCB is rapidly debrominated in whole blood, it appears that MGN is the reactive species responsible for macromolecular binding. From these studies, we conclude that the conversion of BCB to MGN is mediated by a free sulfhydryl-dependent biotransformation pathway. Furthermore, BCB biotransformation is required for erythrocyte binding, and the consumption of free sulfhydryls associated with the biotransformation of BCB is responsible for hemolysis.

BCB1 (CAS: 35691-65-7) is a broad-spectrum microbicidal agent used in a variety of industrial and consumer products. BCB is used to control slime-forming bacterial and fungal growth in industrial water cooling systems, latex paints, metal working fluids, joint cement, and adhesives. In the 1990s, this compound has been used as a preservative in cosmetic products. Several reports have indicated that BCB appears to be a weak skin-sensitizing agent in humans (Van Ginkel and Rundervoort, 1995; Hausen, 1993; Mathias, 1983; Andersen and Rycroft, 1991). The absorption, distribution, metabolism, and elimination of BCB in male Fischer 344 rats were reported by Sauer et al. (1998). They found that no parent compound could be detected in the blood after iv administration of BCB. Its primary metabolite, MGN, however, was detected in blood samples up to 1 hr after iv administration. After 72 hr, a significant portion of the dose was still retained in the blood. This bound fraction in the blood could not be extracted with organic solvents. These results suggest that BCB, or possibly one of its metabolites, covalently binds to constituents of whole blood. Such binding might be a mechanism by which BCB could exert toxicity. The objectives of this study were to elucidate the biotransformation of BCB, characterize its macromolecular binding, and predict the possible toxicological ramifications of such events.

Materials and Methods

Chemicals. BCB was a gift from the Calgon Corp. (Pittsburgh, PA). Radiolabeled [1,3-14C]BCB was obtained from Wizard Laboratories (West Sacramento, CA). [1,3-14C]MGN was synthesized by reacting [1,3-14C]BCB (90 μCi, 7.0 mg) with GSH (34.7 mg), in 1.5 ml of saline, for 3 hr at 37°C. The purity of both [1,3-14C]MGN and [1,3-14C]BCB was 99%, as determined by radiochemical HPLC and GC/MS analysis. MGN, Drabkin’s reagent, GSH, bathophenanthroline disulfonic acid, 2,4-dinitrofluorobenzene, γ-glutamyl transferase, and NEM were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals and reagents used were of the highest purity commercially available.

In Vivo Animal Studies. Male Fischer 344 rats with jugular vein cannulation were purchased from Hilltop Lab Animals (Scottdale, PA). Animals were housed in a facility with a 12-hr light/dark cycle and temperature maintained at 25°C. Animals were acclimated to this environment for a...
minimum of 5 days. They were provided food (Teklad 4% Mouse-Rat Diet; Harlan Teklad, Madison, WI) and water ad libitum. \[^{14}C\]BCB (80 mg/kg, 120 \(\mu\)Ci/kg), in a vehicle of 10% emulphor/15% ethanol/75% saline solution, was administered to male Fischer 344 rats via the indwelling jugular vein cannula. The injection was followed by injection of an equal volume of normal saline solution to flush the cannula. Blood samples (300 \(\mu\)l) were collected at various time points (0, 3, 5, 7, 10, 12, 15, 20, 30, 45, and 60 min and 6, 12, 24, and 48 hr). Immediately after collection, two 25-\(\mu\)l aliquots of blood were assayed for total radioactivity using liquid scintillation counting. Blood samples (150 \(\mu\)l) were also extracted with ethyl acetate and analyzed by HPLC as described below. The remaining portions of the blood samples were centrifuged at 325 \(g\) for 10 min. Plasma (25 \(\mu\)l) and erythrocytes (25 \(\mu\)l) were assayed for \(^{14}C\)-equivalent contents by liquid scintillation counting.

**Biotransformation of BCB in Whole Blood and Buffered GSH Solution.**

In an attempt to elucidate the role of free sulfhydryl groups in the biotransformation of BCB, \[^{14}C\]BCB (4 mg/ml, 60 \(\mu\)Ci/ml, 5 \(\mu\)Ci) was incubated for various times, at 37°C, with fresh rat blood (150 \(\mu\)l) or phosphate (0.2 M, pH 7.4)-buffered GSH (25 mM) solution, with or without NEM (40 mM). At the end of the incubation, samples were treated as described above. Identification of BCB and its metabolites in \textit{in vivo} blood samples was performed by HPLC and GC/MS analysis.

To determine the precise role of GSH in the biotransformation of BCB, quantitative analysis of BCB, MGN, GSH, and GSSG was performed by radiochemical and spectrophotometric HPLC analysis. Incubation mixtures (0.5 ml) containing \[^{14}C\]BCB and GSH, at molar ratios of 1:1, 1:2, and 1:3 (30:30, 30:60, and 30:90 \(\mu\)mol), were incubated at 37°C for 3 hr. After incubation, aliquots of the samples were subjected to radiochemical HPLC analysis, to determine the amounts of BCB and MGN in the mixtures, as described below. The remaining fractions of the samples were analyzed for GSH and GSSG concentrations. Sample preparation and spectrophotometric HPLC analysis were adapted from the methods of Reed et al. (1980). Briefly, samples were mixed with 10% perchloric acid, 1.0 mM bathophenanthroline sulfonic acid, and \(\gamma\)-glutamyl glutamate (internal standard), neutralized with KOH/KHCO\(_3\) (pH 8.5), and derivatized with 1% 2,4-dinitrofluorobenzene. After overnight incubation at 4°C, detection of GSH and GSSG was accomplished using a Beckman HPLC system (model 110B), with an analytical column (Microsorb MV, 4.6 x 250 mm, 5-\(\mu\)m particles) obtained from Rainin Instrument Co. (Woburn, MA). The mobile phase gradient was started using 80% methanol/20% 0.5 M ammonium acetate for 10 min; the mobile phase was then changed to 99.5% 0.5 M ammonium acetate in 5 min, maintained at 99.5% 0.5 M ammonium acetate for 15 min, and returned to 80% methanol/20% 0.5 M ammonium acetate in 5 min, then maintained at 80% methanol/20% 0.5 M ammonium acetate for 15 min. Absorption was measured at 365 nm, with a sensitivity of 0.05 absorbance units full scale.

**Radiochromatograms of BCB and Its Metabolites.** The HPLC analysis of BCB and its metabolites used in this study was described in detail by Sauer et al. (1998). Briefly, aliquots of blood (150 \(\mu\)l) or GSH incubation mixtures were extracted immediately with ethyl acetate (300 \(\mu\)l) three times. The organic solvent was pooled, and 150 \(\mu\)l of distilled water was added. Analytes were pushed into the aqueous phase by evaporation of the ethyl acetate by vacuum centrifugation. Separation and quantification of analytes were performed with an HPLC system (SP8800; Spectra Physics, San Jose, CA) equipped with an autosampler (AS 3000; Thermo Separation Products-Spectra Systems, Fremont, CA) and a \(\beta\)-RAM Model 2 Detector. (with WinFlow; IN/US Systems, Tampa, FL). The samples (100 \(\mu\)l) were injected onto a Whatman Partisil ODS-2 column (250 x 4.6 mm, 10 \(\mu\)m; Whatman, Hillsboro, OR). Analytes were eluted with a mobile phase of water and acetonitrile (both containing 0.1% acetic acid) at a flow rate of 1 ml/min, with a total run time of 80 min. The mobile phase was maintained at 100% water for 5 min and then changed to 20% acetonitrile in 15 min, to 30% acetonitrile in 20 min, and to 100% water in 10 min, with the final conditions being maintained for 10 min. The column was reequilibrated to the initial conditions for 20 min.

MGN was identified using a Fisons GC-8000 gas chromatograph coupled to a Fisons MD800 quadrupole mass spectrometer (Fisons Instruments, Beverly, MA). Analytes (1 \(\mu\)l) were injected onto a DB5-MS capillary column (0.25-\(\mu\)m film thickness, 0.25-mm diameter, 30 \(m\); J&W Scientific, Folsom, CA). The oven temperature was initially maintained at 50°C for 5 min, increased at 10°C/min for the next 25 min, to a final temperature of 300°C, and maintained at 300°C for 5 min. The injector, source, and interface temperatures were set at 280°C and 320°C, respectively. The detector was a Fisons MD800 quadrupole mass spectrometer (Fisons Instruments, Beverly, MA). The oven temperature was initially maintained at 50°C for 4 min, increased at 1°C/min for the next 25 min, to a final temperature of 300°C, and maintained at 300°C for 5 min. The injector, source, and interface temperatures were set at 280°C and 320°C, respectively. The detector was a Fisons MD800 quadrupole mass spectrometer (Fisons Instruments, Beverly, MA).
were 250°C, 250°C, and 275°C, respectively. Mass spectra were scanned from m/z 50 to 650 in 1 sec.

**In Vitro** BCB and MGN Binding Studies. For these studies, whole blood was separated into plasma and erythrocytes by centrifugation at 325 g for 10 min. The erythrocyte fraction was washed with buffered saline (pH 7.4) containing 0.01% CaCl₂, 0.02% KCl, 0.02% KH₂PO₄, 0.0047% MgCl₂, 0.8% NaCl, 0.115% Na₂HPO₄, and 0.1% D-glucose. After three washings, the volume of the packed erythrocytes was adjusted with buffered saline. Hemoglobin was prepared according to the method of Zulstra and Buursma (1987).

[¹⁴C]BCB (4 mg/ml, 60 μCi/ml) or [¹⁴C]MGN (1.65 mg/ml, 60 μCi/ml) was mixed with whole blood, plasma, isolated erythrocytes, or purified hemoglobin (150 μl) and incubated, with or without NEM (40 mM), at 37°C for 15, 30, and 60 min and 2, 4, 6, and 8 hr. Samples were repeatedly extracted with ethyl acetate, until the level of radioactivity in the ethyl acetate was less than the background levels. [¹⁴C]-equivalents in pooled ethyl acetate extracts were determined by liquid scintillation counting. The percentage of binding for each time point was calculated according to the following formula: % of binding = (total dose − extracted [¹⁴C]-equivalents) / total dose × 100.

**Spectral Effects of BCB and MGN on Purified Hemoglobin.** Hemoglobin was isolated and purified from rat blood according the method of Zulstra and Buursma (1987). Freshly isolated hemoglobin (150 μl) was incubated with various concentrations of BCB or MGN (0.75–3.05 mM) at 37°C for 15 min. After incubation, sample absorbance was scanned from 450 to 750 nm using a Beckman DU-7 spectrophotometer, with a normal bandwidth of 0.1 nm and light path of 1.0 cm.

**Statistical Analysis of Data.** Experiments were performed at least three times, and sample size refers to the number of animals. Data are presented as the arithmetic mean ± standard deviation. Statistical analysis of the data for more than two groups was conducted by repeated-measures one-way analysis of variance followed by the Newman-Keuls test. The paired Student test was used to analyze the data for two groups. Differences were considered to be significant at p < 0.05.

**Results**

**In Vivo Animal Studies.** After iv administration of [¹⁴C]BCB, HPLC analysis of blood samples revealed MGN as the single detectable analyte in the blood. No parent compound was detected in the blood even at the earliest time point of 3 min. After administration, the percentage of [¹⁴C]-equivalents in the blood increased gradually over time and consistently represented >5% of the total dose after 1 hr (fig. 1). After 48 hr, approximately 12% of the dose was covalently associated with the blood. The plasma/erythrocyte disposition ratio for covalently bound [¹⁴C]-equivalents was 1.4.

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**TABLE 1**

Stoichiometric determination of GSH-dependent biotransformation of BCB in *in vitro* incubations of BCB with different concentrations of GSH

<table>
<thead>
<tr>
<th>BCB/GSH Molar Ratio</th>
<th>BCB (μmol)</th>
<th>MGN (μmol)</th>
<th>GSH (μmol)</th>
<th>GSSG (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>13.9</td>
<td>16.2</td>
<td>bql*a</td>
<td>15.3</td>
</tr>
<tr>
<td>1:2</td>
<td>bql</td>
<td>30.4</td>
<td>1.04</td>
<td>30.1</td>
</tr>
<tr>
<td>1:3</td>
<td>bql</td>
<td>29.8</td>
<td>29.4</td>
<td>29.9</td>
</tr>
</tbody>
</table>

Samples were incubated at 37°C for 3 hr; 30 μmol of BCB was added to initiate the reaction. BCB and MGN were determined with a HPLC radiochromatographic assay (fig. 5), and GSH and GSSG were determined with a HPLC spectrophotometric assay (fig. 6).

*a* bql, below quantitative limit.

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The mass spectrum showed a molecular ion corresponding to m/z 106. The major product ion was at m/z 66, which represents a loss of 40 mass units for the methylenecyanide radical.

**FIG. 3.** Representative GC/MS electron impact spectrum of MGN obtained from an incubation of [¹⁴C]BCB with blood.

**Spectral Effects of BCB and MGN on Purified Hemoglobin.** Hemoglobin was isolated and purified from rat blood according the method of Zulstra and Buursma (1987). Freshly isolated hemoglobin (150 μl) was incubated with various concentrations of BCB or MGN (0.75–3.05 mM) at 37°C for 15 min. After incubation, sample absorbance was scanned from 450 to 750 nm using a Beckman DU-7 spectrophotometer, with a normal bandwidth of 0.1 nm and light path of 1.0 cm.

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**Statistical Analysis of Data.** Experiments were performed at least three times, and sample size refers to the number of animals. Data are presented as the arithmetic mean ± standard deviation. Statistical analysis of the data for more than two groups was conducted by repeated-measures one-way analysis of variance followed by the Newman-Keuls test. The paired Student test was used to analyze the data for two groups. Differences were considered to be significant at p < 0.05.
from GSH. When BCB was incubated with GSH in a 1:1 molar ratio (30:30 \(\mu\)mol), approximately one half (13.9 \(\mu\)mol) of the BCB in the incubation remained unchanged, whereas GSH concentrations were below the quantitative limit of the assay. The reaction generated 16.2 \(\mu\)mol of MGN and 15.3 \(\mu\)mol of GSSG (table 1, fig. 4). When BCB and GSH were incubated at a 1:2 ratio, all of the BCB in the reaction mixture was converted to MGN and very little GSH escaped conversion to GSSG. When BCB and GSH were incubated at a molar ratio of 1:3, BCB was completely converted to MGN (29.8 \(\mu\)mol). With this ratio, GSH was at a molar excess (~30 \(\mu\)mol), and the amounts of GSSG (29.9 \(\mu\)mol) and GSH (29.4 \(\mu\)mol) in the solution were nearly identical after incubation. The results of these experiments indicate a reaction formula in which two GSH molecules are required to convert one BCB molecule to one MGN molecule, resulting in the formation of GSSG.

**In Vitro BCB and MGN Binding Studies.** The percentage of binding for BCB and MGN incubated with whole blood, erythrocytes, and hemoglobin is shown in table 2. As the incubation time was increased, the amounts of BCB and MGN bound to whole blood and erythrocytes increased progressively. Coincubation of whole blood and erythrocyte preparations with NEM resulted in significantly decreased binding ratios for both BCB and MGN (fig. 5). Both BCB and MGN showed covalent binding (approximately 30%) to purified hemoglobin. In the other preparations, binding to hemoglobin could be inhibited with NEM.

**Spectral Effects of BCB and MGN on Purified Hemoglobin.** Purified hemoglobin from isolated rat erythrocytes showed a typical hemoglobin spectrum, with \(A_{\text{max}}\) values of 540 and 574 nm (fig. 7). However, the intensity of the absorbance spectrum declined with increasing concentrations of BCB (0.75–3 mM). With MGN, there was no alteration of the hemoglobin spectrum at any of the concentrations tested.

**Discussion**

After iv administration, BCB is labile, being rapidly debrominated to form MGN. A progressively larger amount of \(^{14}\)C-equivalents become bound to blood constituents. Furthermore, approximately 80% of the bound \(^{14}\)C-equivalents were associated with the erythrocyte fraction of the blood and could not be displaced by extraction with various solvents. These initial observations raise two questions, as follows: what is the mechanism of the rapid conversion of BCB to MGN and what is the chemical entity (BCB or its metabolites) that binds to erythrocytes? To answer these questions, further studies focused on characterizing the biotransformation of BCB and its ability to covalently bind to macromolecules.

Sauer et al. (1998) showed that whole blood debrominates BCB. This rapid conversion by whole blood is likely responsible for the absence of detectable BCB after iv administration. Furthermore, it was speculated that this conversion is mediated by free sulfhydryls, analogously to a metabolic scheme described by Livesey et al. (1982). To further characterize BCB biotransformation, BCB was incubated...
with plasma, erythrocytes, or whole blood obtained from male Fischer 344 rats. The results showed that BCB was converted to MGN by erythrocytes and whole blood in <30 sec. However, conversion by plasma was much slower than that by erythrocytes or whole blood. Erythrocytes, as well as plasma, contain high concentrations of both protein and nonprotein sulfhydryl groups, which can interact with xenobiotics (erythrocytes >> plasma) (Tirumalai et al., 1996; Connor and Schroit, 1990). To investigate the possible role of sulfhydryl groups in the conversion of BCB to MGN, buffered GSH solutions were incubated with BCB. As in incubations with blood, BCB was readily debrominated and converted to MGN. Furthermore, the addition of NEM, a sulfhydryl-alkylating agent, to whole blood and GSH solutions blocked the conversion of BCB to MGN. The reactivity of MGN toward sulfhydryl groups is further illustrated by the urinary metabolites of BCB. The primary urinary metabolite of BCB results from the conjugation of MGN with GSH and is a mercapturic acid conjugate of MGN [N-acetyl-S-(2,4-dicyanobutane)-L-cysteine] (Sauer et al., 1998). Thus, the presence of sulfhydryl-containing molecules is required for the conversion of BCB to MGN, as well as the further metabolism of MGN.

The role of sulfhydryls in the biotransformation of BCB was further elucidated when it was determined that two molecules of GSH were

\[\text{FIG. 5. Time course for covalent binding of BCB and MGN, with and without NEM coincubation, in whole blood, isolated erythrocytes, and isolated hemoglobin.} \]

Data are expressed as mean ± SD. Samples from four individual rat preparations were analyzed in duplicate. **, p < 0.01, compared with NEM-treated groups.
required to convert one molecule of BCB to MGN. During the reaction, the two GSH molecules are oxidized to form GSSG. This evidence strongly suggests that there is a quantitative relationship between the conversion of BCB to MGN and the formation of GSSG from GSH. Indeed, as speculated by Sauer et al. (1998), the debromination of BCB is mediated by a free sulfhydryl-dependent biotransformation pathway. The nucleophilic attack by GSH does not result in the formation of a stable GSH conjugate. Instead, two GSH molecules are oxidized to GSSG. This reaction mechanism is similar to that previously described by Livesey et al. (1982), in which the nucleophilic attack of GSH on a dihaloalkyl substrate results in S-(β-haloalkyl)-GSH formation; subsequent attack of a second thiol on the sulfur atom of the conjugate yields GSSG, two halide ions, and an alkene product.

Incubation of BCB or MGN with whole blood or isolated erythrocytes resulted in progressive binding, in which 75–90% of the 14C-equivalents were bound after 8 hr of incubation. BCB and MGN showed nearly identical kinetic binding profiles. These data, along with the biotransformation data discussed above, suggest that MGN is responsible for the observed macromolecular binding. In addition, there may be specific binding sites or targets for MGN in whole blood and on erythrocyte membranes. NEM, which has been shown to prevent the conversion of BCB to MGN, also reduces the amount of macromolecular binding of both BCB and MGN in whole blood and erythrocyte incubations. Thus, in the case of BCB, it is clear that NEM inhibits biotransformation and only a limited amount of binding occurs. However, for MGN, sulfhydryls are not involved in further metabolism but NEM inhibits binding apparently by competing for common binding sites. Therefore, we conclude that MGN is the reactive species responsible for macromolecular binding and that free sulfhydryl groups are the targets for MGN binding.

Another significant observation was that BCB incubation with erythrocytes resulted in hemolysis, in a dose-related fashion. After 2 hr of incubation, the cytotoxicity curve was fitted to a sigmoidal profile and an EC50 of 1.0 ± 0.3 mM was calculated. Additionally, this dose-response relationship could be altered by coincubation with NEM. Although a 0.5 mM concentration of NEM did not cause...
hemolysis during the 2-hr incubation period, a synergistic hemolytic response was observed with NEM in combination with BCB. In contrast, coincubation with GSH protected erythrocytes from BCB-induced hemolysis. As previously discussed, the debromination of BCB is mediated by the oxidation and consumption of free sulfhydryls, including GSH. Other xenobiotics that interact with sulfhydryl groups can cause significant physiological or biochemical changes in erythrocytes. For example, several investigators have reported that the oxidation of membrane-associated sulfhydryl groups by diamide and 2,3-diprophosphoglycerate results in erythrocyte membrane rigidity and enhanced permeability to hydrophilic nonelectrolytes and ions (Klonk and Deuticke, 1992; Chasis and Mohandas, 1986). These changes were specific to the oxidative modifications of sulfhydryl groups and were reversible upon reduction back to the free sulfhydryls. Lauriault and O’Brien (1991) also reported that disulfiram-induced hemolysis was markedly enhanced in GSH-depleted erythrocytes. This rapid hemolysis involves the oxidation of plasma membrane-associated protein sulfhydryl groups in lysis. It is of interest to note that MGN does not cause hemolysis of erythrocytes even at a concentration that is 10-fold higher than the calculated EC₅₀ for BCB. Thus, it appears that BCB-induced hemolysis results from the oxidation of sulfhydryls, as well as the consumption of GSH, and not from macromolecular binding (fig. 8). However, it is important to note that hemolysis was not observed in the in vivo studies at the doses used in previous studies (data not shown). The explanation for this difference likely involves the concentrations of GSH available to the erythrocytes in vivo, compared with the limited amounts present in in vitro incubations, which can be readily consumed during the conversion of BCB to MGN.

Both BCB and MGN covalently bind to purified hemoglobin, at approximately the same levels (30%). However, unlike the other in vitro preparations, hemoglobin does not catalyze the conversion of BCB to MGN (data not shown). In this case, it appears that BCB binding is not mediated through MGN but, instead, BCB itself binds hemoglobin. Although the binding associated with both of these compounds could be reduced with NEM, there appear to be significant differences in the binding of BCB and MGN to hemoglobin. This difference is evident in a comparison of the abilities of BCB and MGN to alter the spectrum of hemoglobin. With increasing concentrations of BCB, the absorbance spectrum for hemoglobin was gradually reduced, whereas there were no spectral changes after incubation with MGN at any of the concentrations tested.

In summary, BCB is rapidly debrominated to form MGN in the blood, via a free sulfhydryl-dependent biotransformation pathway. This metabolic route results in the consumption of two GSH molecules for each molecule of BCB converted to MGN. This free sulfhydryl-exhausting pathway also results in the loss of erythrocyte viability, mediated by oxidation of free sulfhydryls in the erythrocyte membrane. BCB and its metabolite MGN appear to be the reactive species responsible for macromolecular binding. Although systemic exposure of rats to [¹⁴C]BCB resulted in significant binding of ¹⁴C-equivalents to erythrocytes and other blood constituents, there was no overt in vivo cytotoxicity observed with this acute exposure. This may be the result of the immediate biotransformation of the limited amount of BCB in the systemic environment. However, the oxidative stress induced by BCB in erythrocytes cannot be considered unimportant, when it is viewed as an initiating event in a toxic interaction with another toxicant, which also consumes sulfhydryls or requires GSH for detoxification. Furthermore, BCB acting as a systemic sensitizing agent, thus mediating a hypersensitivity response, remains a possible mechanism of toxicity and warrants further study.

We thank Drs. H. B. Matthews and M. L. Cunningham of the National Toxicology Program, as well as L. T. Rael, for advice and support with these studies. We also thank Dr. R. J. Huxtable for reviewing the manuscript and giving advice.

References