LETTERS TO THE EDITOR

Hemoglobin Adducts of Monohalomethanes

Key words: Methyl chloride—Methyl bromide—Methyl iodide—Hemoglobin

Determination of covalent binding to hemoglobin (Hb) has been proposed as a means of biomonitoring of exposure to carcinogenic and mutagenic substances.^{1,2)} In a recent letter to this journal, Iwasaki et al.³⁾ have applied this methodology to workers exposed to methyl bromide. We want to extend these studies by comparing the alkylation of Hb in rats by the three monohalomethanes methyl chloride, methyl bromide and methyl iodide.

No covalent binding of methyl chloride to DNA was found in previous experimental studies (Kornbrust et al.;⁴⁾ Peter et al.⁵⁾). In contrast, DNA adducts in different organs of rats (F-344) exposed to methyl bromide and methyl iodide suggest systemic genotoxic potentials of these two closely related chemicals.⁶⁾

Oral exposures: Nine μmol of ^{14}C -labelled chemical (methyl bromide or iodide), dissolved in neutral oil, was administered to each of five male and five female rats by gavage.

Inhalative exposures: Five male and 5 female F-344 rats were exposed to methyl bromide, methyl iodide or methyl chloride (males only) in the closed exposure system described by Bolt et al.⁷⁾ For each exposure, 45 μmol test chemical (^{14}C -labelled) was injected into the system, except for the exposure of female rats to methyl bromide, in which 90 μmol was applied.

The inhalation exposures were terminated after 24 hours. The animals were sacrificed at this time point (inhalation exposures) or 6 hours after the oral gavage, respectively. Blood was collected in heparinized tubes. Erythrocytes were sedimented by centrifugation and washed twice with saline. Erythrocytes (0.5 ml) were lysed in 2 ml 0.4 mmol EDTA. Cell debris was removed by centrifugation at 4000 g for 15 minutes. Hb was determined with the cyanomethemoglobin method (Hb determination kits from Sigma).

Globin was separated and purified by acidic acetone precipitation and pre-treated for liquid scintillation counting as described by Pereira and Chang⁸⁾ with some modifications: 2 ml solution (containing about 10 mg Hb) was added dropwise to 20 ml 1% HCl-acetone; supernatant was removed by centrifugation at 15000 g; the globin was washed successively with 1% HCl-acetone and acetone. The globin was then dissolved in 0.5 ml bidistilled water, and aliquots were taken

Table 1. "Covalent binding indices" of monohalomethanes to hemoglobin.
The figures give the amount of methyl halide bound to Hb,
related to the total dose given in F-344 rats:

		pmol bound/mg Hb			
		μ mol administered/kg b.w.			
methyl chloride		methyl bromide		methyl iodide	
	oral	oral	inhalative	oral	inhalative
M	145	871	1, 335	248	976
	± 16	± 139	± 54	± 30	± 91
F	n.d.	538	1, 670	229	1, 059
		± 149	± 99	± 20	± 90

n.d.=not determined

$X \pm S.D.$ are given. $n=5$ rats in each experiment.

for quantitation of globin protein according to Lowry *et al.*⁹⁾ The remainder was mixed with 0.5 ml TS-1: ethanol (1:2) and incubated at 60°C for 1 hour in a sealed scintillation vial. 0.5 ml tert-butylperoxide was then added to decolorise the solution. The solution was neutralised afterwards with 0.5 ml 0.5 N HCl and mixed with Quickszint 2000. Radioactivity was counted in a TriCarb 2200-Ca liquid scintillator.

Table 1 gives the relative binding figures of the three monohalomethanes to Hb, expressed as "covalent binding indices" (i.e., covalently bound material, related to administered dose). No major differences between both sexes of F-344 rats appeared. Interestingly, the highest Hb binding was observed with methyl bromide. This might be explained by a lower reactivity of methyl chloride on one hand, and a higher detoxification rate (due to higher reactivity) of methyl iodide, on the other.

Moreover; higher binding figures to Hb were observed when methyl bromide or methyl iodide were administered via inhalation, compared to the oral route. This points to a first-pass-effect: both compounds (methyl bromide and methyl iodide) may partly be detoxified on their first liver passage which prevents them from alkylating Hb.

These two features, leading to an especially high binding of inhaled methyl bromide to Hb, very much support the suggestion of Iwasaki *et al.*³⁾ that the Hb adduct of methyl bromide may be a useful parameter in biological exposure monitoring of methyl bromide workers.

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