ABSTRACT

Infestation of flour and other stored products by the tenebrionid (flour beetles) has been proven a health hazard to the public. Secretions from these insects have been found to be harmful toxicologically. These secretions also possess characteristics typical of mutagens. Therefore the mutagenic potential of 2-methyl-1, 4-benzoquinone (MBQ), 2-ethyl-1, 4 benzoquinone (EBQ) and 1-pentadecene (P-dec) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay, a screening test for detection of mutagens. Tester strains TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses of $10^{-1}$ mg/plate to $3.2 \times 10^{-5}$ mg of MBQ/plate, $10^{-2}$ mg/plate to $3.2 \times 10^{-6}$ mg of EBQ/plate and 1 mg/plate to $3.2 \times 10^{-2}$ mg of P-dec/plate. No evidence of mutagenic potential was observed at the levels tested.

Infestation of flour and stored products by members of the Tenebrionidae (Coleoptera) has been a problem in this country from both economic and health standpoints. Beetles of the Tribolium spp. are the most common of the tenebrionid pests. Secretions isolated from these insects have been found in sufficient quantities in stored products to be considered a hazard to the public (3). Larger beetles contain mean values of 228 \mu g of EBQ plus MBQ per insect (7). These secretions consist of 2-methyl-1, 4-benzoquinone (MBQ), 2-ethyl-1, 4 benzoquinone (EBQ) and 1-pentadecene (P-dec).

The Armed Forces have unique requirements, often under undesirable environmental conditions, for long-term storage and transportation of subsistence. The objective of this study was to assess the hazard presented to soldiers consuming infested food products.

Several toxicological assays have been performed on these compounds (6). The LD$_{50}$ in rats was determined to be 145 mg/kg for MBQ, 205 mg/kg for EBQ, and greater than 10 g/kg for P-dec (5). Studies examining the effects of the substituted benzoquinones on red blood cell heme and hepatic microsomal heme demonstrated significant deleterious alterations (4). These molecules have a low molecular weight and possess alkylating and arylating properties. These characteristics are consistent with those of mutagens. To determine the mutagenic potential of these compounds, the Ames Salmonella/Mammalian Microsome Mutagenicity Assay was performed (1). Exposures ranged from the highest sublethal dose to a greater than 1000-fold dilution. Compounds were assayed both in the presence and absence of the liver microsomal enzymes, referred to as S-9. This metabolic activation is believed to mimic mammalian metabolism.

MATERIALS AND METHODS

Experimental methods followed were those cited in the Ames Methods paper (1). The bacterial strains (TA 98, TA 100, TA 1535, TA 1537, and TA 1538) were provided by Dr. Bruce Ames, University of California, Berkeley. MBQ was obtained from Baker Chemical Company (Phillipsburg, NJ 08865) and was dissolved in dimethyl sulfoxide (DMSO). P-dec was obtained from Sigma Chemical Company (St. Louis, MO 63178) and was dissolved in ethanol. EBQ was synthesized by the method of Ladisch and Suter (2) and dissolved in DMSO. The S-9 liver microsomal enzyme fraction was obtained from Litton Bionetics (Kensington, MD 20795), and the optimal concentration was found to be 0.75 mg of protein/plate. The toxicity level determination was made to establish a sublethal dose. From the highest sublethal concentration, six five-fold dilutions were made to determine the presence of a correlated dose response. The plate incorporation method was followed.

RESULTS AND DISCUSSION

Following the toxicity level determination, we decided to use $10^{-1}$ mg of MBQ/plate, $10^{-2}$ mg of EBQ/plate, and 1 mg of P-dec/plate as the initial dose. Six five-fold dilutions were made down to $3.2 \times 10^{-5}$ mg/plate, $3.2 \times 10^{-6}$ mg/plate, and $3.2 \times 10^{-4}$ mg/plate for MBQ, EBQ and P-dec, respectively. For a mutagenic response in the Ames Assay, one must observe more than a doubling of the spontaneous reversion rate and a correlated dose response (1). Our data showed no such evidence (Table 1).
TABLE I. Response of the Ames tester strains to the substituted benzoquinones. The value for each dilution was determined as the average of triplicate plate counts. Numbers listed are the average of all dilutions used.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Dose (mg/plate)</th>
<th>TA 98</th>
<th>TA 100</th>
<th>TA 1535</th>
<th>TA 1537</th>
<th>TA 1538</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBQ (^{1})</td>
<td>(10^{-3}) 3.2 (\times) (10^{-5})</td>
<td>18</td>
<td>25</td>
<td>105</td>
<td>110</td>
<td>14</td>
</tr>
<tr>
<td>Spontaneous reversion</td>
<td>26</td>
<td>21</td>
<td>104</td>
<td>96</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>P-dec (^{b})</td>
<td>1 to 3.2 (\times) (10^{-4})</td>
<td>20</td>
<td>25</td>
<td>98</td>
<td>91</td>
<td>16</td>
</tr>
<tr>
<td>Spontaneous reversion</td>
<td>26</td>
<td>21</td>
<td>104</td>
<td>96</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>EBQ (^{c})</td>
<td>(10^{-2}) to 3.2 (\times) (10^{-6})</td>
<td>16</td>
<td>25</td>
<td>87</td>
<td>91</td>
<td>13</td>
</tr>
</tbody>
</table>

\(^{a}\)2-methyl-1,4-benzoquinone.  
\(^{b}\)1-pentadecene.  
\(^{c}\)2-ethyl-1,4-benzoquinone.  
\(^{d}\)O= No S-9.  
\(^{e}\)S-9 = With S-9 microsomal enzymes.

CONCLUSION

TA 98, TA 1537 and TA 1538 function to determine the presence of frameshift mutagens. TA 100 and TA 1535 are used to identify basepair mutagens. Due to the negative response in all these strains we can find no evidence for mutagenic potential. Therefore, on the basis of the Ames Assay, the substituted benzoquinones are not mutagenic at the levels tested.

REFERENCES