Mutagenicity of Tenebrionid Flour Beetle Secretions Using Drosophila melanogaster Sex-Linked Recessive Lethal Test

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ABSTRACT

The Tribolium spp. beetles are the most common tenebrionids infesting flour and other stored foods. 2-Ethyl-1,4-benzoquinone (EBQ) and 2-methyl-1,4-benzoquinone (MBQ) are the major secretory products of these insects. Benzoquinones are highly reactive compounds which have been reported to be acutely toxic and carcinogenic to laboratory animals. Using the Drosophila melanogaster sex-linked recessive lethal test, we examined the mutagenicity of EBQ and MBQ. Feeding concentrations of 1 mM EBQ and 2 mM MBQ in 1% sucrose resulted in 72-h mortalities of 25% for EBQ and 39% for MBQ in adult Canton-S male flies. A comparable mortality rate for negative control insects fed 1% sucrose was 2.5%, while positive control flies fed 1 mM ethyl methanesulfonate (EMS) in 1% sucrose resulted in a 2.3% mortality rate. Mutation rates resulting from these exposure levels are as follows: negative control, 0.03%; positive control, 16.05%; 1 mM EBQ, 0.16%; and 2 mM MBQ, 0.13%. The mutation rates for flies fed EBQ and MBQ were significantly higher (p<0.005 for EBQ and p<0.016 for MBQ) than those of concurrently tested negative control insects when analyzed by both the Fisher’s exact and Kastenbaum-Bowman tests. These results show EBQ and MBQ to be mutagenic when tested using the sex-linked recessive lethal Drosophila melanogaster system. Analysis of brood mutation rates indicate that both EBQ and MBQ act as indirect mutagens. The presence of benzoquinone-secreting Tribolium spp. flour beetles in food products could represent a toxicologic hazard to the consumer. Presently no distinction is made between benzoquinone-secreting insects and other arthropods infesting stored products when establishing rejection standards for infested foods.

The presence of arthropods in food products has usually been viewed as unacceptable from an aesthetic viewpoint (8). There is also concern that insects may present potential toxicologic problems due to the secretory and excratory products they may release into stored commodities. While several food-infesting arthropods can be identified by their odor (23), only in the tenebrionid flour beetles have the major secretory products been isolated and identified (1,15). The Tribolium spp. beetles are the most common tenebrionids infesting flours and other stored products. These insects possess glands in the thorax and abdomen that secrete an odoriferous mixture of substituted p-benzoquinones and short-chain hydrocarbons. 2-Ethyl-1, 4-benzoquinone (EBQ), 2-methyl-1, 4-benzoquinone (MBQ), and 1-pentadecene are the major secretory products of several common species (26,27). The larger beetles contain mean levels of 228 to 255 μg of EBQ plus MBQ per insect (27).

EBQ and MBQ are highly reactive compounds and evidence indicates they are acutely toxic and perhaps carcinogenic to laboratory animals. The acute lethal dose (LD₅₀) of the hydroquinone - quinone system ranges from 50 to 300 mg/kg body weight (18,19). Gastric intubation of a single 30 to 50 mg/kg dose of EBQ-MBQ mixture resulted in high mortality and a high incidence of sweat gland carcinoma in mice (13). Benzoquinones have been described as bactericidal, fungistatic, spermicidal, enzyme-inhibiting, antimitotic, carcinogenic and mutagenic (9). Using the Drosophila melanogaster sex-linked recessive lethal (SLRL) test, we examined EBQ and MBQ and found them to be mutagenic at feeding concentrations of 1 to 2 mM.

MATERIALS AND METHODS

Test and control chemicals

Ethyl methanesulfonate (EMS) was purchased from Fisher Scientific Co., Santa Clara, CA, and MBQ from J. T. Baker Chemical Co., Phillipsburg, NJ. EBQ was synthesized following the procedures of Ladisch and Suter (11). The synthetic EBQ exhibited only one peak when subjected to gas-liquid chromatography (28) and had a sharp melting point of 38.7±0.2°C.

Drosophila melanogaster stocks

The D. melanogaster strains used for SLRL testing were obtained from Dr. R. Valencia, Zoology Research Laboratory, University of Wisconsin, Madison, WI. The Canton-S (CS) stock is a wild-type strain, with round red-eyed males, which exhibits a relatively low constant spontaneous mutation frequency (0.1 to 0.2%). The First Multiple Number 6 (FM6) insects contain a homozygous X-chromosome which carries phenotypic markers for yellow body (y), bar-shaped eye (B) and white-colored eye (w), and several superimposed structural inversions which prevent

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"crossing over" (exchange of parts) with a homologous non-inverted X-chromosome.

All insects were reared at 22 ± 2°C and 50 ± 10% relative humidity with a 12:12-h photoperiod. Preparation of the cornmeal-based medium used for rearing all stock and experimental insects is described by Wirtz and Semy (25).

**SLRL testing**

Adult CS males, collected approx. 24 h after pupal eclosion, were exposed to a control or test solution for 72 h to ensure feeding. Mortality rates of 99 to 100% were observed for flies that were not allowed to feed for 3 d. Ten CS males were transferred to each glass feeding vial (24 × 95 mm) containing a 2.1- and a 2.4-cm diameter glass microfiber disc (Whatman GF/A) tamped to the bottom of the vial, saturated with 0.25 ml of the control or test solution, and stoppered with a rayon plug. Flies were transferred to a vial containing fresh solution every 24 h. Feeding concentrations of test chemicals were 1 mM EBQ and 2 mM MBQ in 1% sucrose. Negative control insects received 1% sucrose, whereas positive control insects were fed 1 mM EMS in 1% sucrose.

After treatment, each male was assigned a unique sequential number (6) and mated to three FM6 virgin females (Brood 1) in a vial containing rearing medium. The males were transferred to new groups of three FM6 virgin females after 3-, 2-, and 2-d intervals (Broods 2, 3, 4, respectively) and removed from Brood 4 females after an additional 3 d. This brooding sequence was used to sample germ cells in different stages of germ formation during exposure to test chemicals (28).

After pupal eclosion of the F1 progenies from the four broods, the bar red-eyed daughters were mated individually to their male bar white-eyed siblings. The resulting F2 progenies were examined for the presence or absence of normal appearing male flies. The absence of this phenoctic class of males with round red eyes indicated that a mutation to a lethal state had occurred in one or more loci on the X-chromosome. F2 confirmation crosses were done in triplicate on all lethals from test- and control flies exposed to 1 mM EMS in 1% sucrose was 15.6 ± 1.9% (mean ± s.d., n = 7, total lethals = 958, total tests = 5,970).

Results of the SLRL mutagenicity tests are given in Table 1. The negative control spontaneous mutation rate of 0.03% is much lower than the reported range of 0.1 to 0.2% for the same strain of CS males reared at the Madison laboratory. This may be the result of differences in medium preparation or ingredient sources. The mutation rate for concurrently tested positive control insects fed 1 mM EMS in 1% sucrose was 2.3%.

Mutation rates of flies fed EBQ and MBQ were significantly higher (p<0.005 for EBQ and p<0.016 for MBQ, Table 1) than spontaneous rates when analyzed by both the Fisher’s exact (2) and Kastenbaum-Bowman (7) tests. These results indicate that EBQ and MBQ were mutagenic when tested using the SLRL D. melanogaster system.

The recorded mutations (Table 1) for insects fed negative control and test compounds occurred in the following broods: control - Broods 1 and 2, 1 and 3 mutations, respectively; 1 mM EBQ: Broods 2, 3, 4, 1, 7 and 2 mutations, respectively; and 2 mM MBQ: Broods 2, 3 and 4, 2 and 2 mutations, respectively. Pooling the brood data into matings A: 1 to 3 d and matings B: 4 to 10 d after exposure, resulted in the following: 1 mM EBQ - matings A and B, 0 and 10 mutations, respectively, and 2 mM MBQ - matings A and B, 0 and 8 mutations respectively. The rationale for pooling the brood data was that in mating groups A, metabolically inactive cells were tested which were easily mutated (e.g., by direct-acting alkylating or cross-linking agents) (21,22). In mating groups B, metabolically active cells were tested which were able to activate a large variety of indirect mutagens (21,22), and which contained active DNA repair systems (W. R. Lee, personal communication). Based on this, both EBQ and MBQ appear to be indirect mutagens.

**RESULTS AND DISCUSSION**

The feeding concentrations of 1 mM EBQ and 2 mM MBQ in 1% sucrose resulted in 72-h mortalities of 25.3% for EBQ and 38.5% for MBQ. The exposure levels are lower than desired, the recommended levels being 3% for EBQ and 38.5% for MBQ. The exposure levels are lower than desired, the recommended levels being 3% for EBQ and 38.5% for MBQ. These results indicate that EBQ and MBQ were mutagenic when tested using the SLRL D. melanogaster system.

<table>
<thead>
<tr>
<th>Feeding solutions</th>
<th>Total lethals</th>
<th>Total tests</th>
<th>Percent lethals</th>
<th>Fisher’s exact</th>
<th>Kastenbaum-Bowman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - 1% sucrose</td>
<td>4</td>
<td>13,068</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 mM EBQ in 1% sucrose</td>
<td>10</td>
<td>6,759</td>
<td>0.15</td>
<td>0.0048</td>
<td>0.0049</td>
</tr>
<tr>
<td>2 mM MBQ in 1% sucrose</td>
<td>8</td>
<td>6,255</td>
<td>0.13</td>
<td>0.0155</td>
<td>0.0156</td>
</tr>
</tbody>
</table>

*aAll single mutations, i.e., no ‘‘clusters’’ or ‘‘multiples’’ were detected.

*bBerchtold (2) and Kastenbaum and Bowman (7) (EBQ: K = 0.34089, M = 14; MBQ: K = 0.32370, M = 12).
reported mutagenic activities of substituted \( p \)-benzoquinones; however, mutagenesis was attributed to the reactive groups attached to the benzoquinones.

Benzoquinones are easily transformed to hydroquinones which impact negatively on biological systems undergoing mitosis (14). Quinones are highly reactive compounds which form conjugates with the -NH and -SH groups of amino acids, proteins and similar compounds (10, 12). The toxicity of these conjugates remains to be determined; however, Ladisch and Suter (12) have suggested that this affinity for amino acids and proteins is responsible for the toxic and carcinogenic properties of benzoquinones. Because of the high level of food infestation attributed to the \( T\)ribolium spp. flour beetles, their secretion of potentially toxic, mutagenic and carcinogenic quinones and the resulting quinone conjugates into food products could represent a considerable toxicologic hazard. Present analytical methods for detecting insect infestations are usually based upon actual insect counts, insect fragments, or damaged kernels (3), and no distinction is made between quinose-secreting insects and other stored-products arthropods (5). An exception to this policy is followed by the Armed Forces who stipulate that three or more \( T\)ribolium spp. beetles per pound of infested commodity is justification for condemnation of the food product (4).

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REFERENCES